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THE ONE-HOUR TWO-DOSE DEXTROSE TOLERANCE TEST*

WILLIAM G. EXTON AND ANTON R. ROSE

From the Laboratory and Longevity Service of The Prudential Insurance Company of America, Newark, New Jersey

Alimentary glucose tolerance tests are in general use because they tell more about the way an organism handles carbohydrates than other clinical methods. Like them, however, sugar tolerance tests also have limitations which are very real, and current clinical opinion concerning their diagnostic value may, perhaps, best be portrayed by a few quotations from contemporary authorities. Thus Joslin states: "Glucose and other food tolerance tests are often unreliable." Mosenthal says: "Increase of glycemia following the ingestion of sugar may be of value in diagnosing diabetes in some cases." Wilder writes: "Atypical responses which are difficult to evaluate are sometimes obtained with the dextrose tolerance test," and Rabinowitch points out: "With blood sugar time curves, as with other laboratory tests, a variety of conditions may be responsible for similar results."

Even more impressive than this unanimity of opinion is the prevailing diversity of practise in making sugar tolerance tests, and this involves not only laboratory technics. These are, in fact, unimportant in comparison with the existing differences in such practical details as preventing glucolysis, timing the tests, collecting the blood and urine samples, preparing and administering glucose and giving different amounts of glucose. Thus, the answers of one hundred clinical laboratories to a questionnaire about these details indicated in 1931 that it was very doubtful if any two of the laboratories were performing tests in exactly the same way.

The literature of sugar tolerance tests is extensive and dates

* Read before the Thirteenth Annual Convention of the American Society of Clinical Pathologists, Cleveland, Ohio, June 8 to 11, 1934.

back many years, but there is nothing in it to suggest that differences of opinion concerning the physiology involved account for either the diagnostic limitations or variations in procedure. On the contrary, the principles are well understood and there is no disagreement about them. On the other hand, it is plain that the diagnostic limitations of sugar tolerance tests flow from their frequent failure to yield definite or dependable results. It is also clear that the diversity of practise is only evidential of individual efforts to secure results that are more specific in their meaning than atypical or doubtful blood sugar time curves which are at best difficult to interpret and often misleading.

FACTORS WHICH AFFECT THE SPECIFICITY OF SUGAR TOLERANCE TESTS

In a previous paper we[3] have considered some of the reasons why the results of sugar tolerance tests so often lack specificity for the particular diminished carbohydrate tolerance of diabetes, and have also presented data of experiments intended to explore the possibilities of improving their specificity. In that paper we considered the test as a physiological experiment which at some definite time measured the difference between the rate at which ingested sugar enters the blood and the rate at which the same body either oxidizes, stores or excretes it. As such measurements are, of course, affected or vitiated by complicating conditions which are more or less uncontrollable, like diuresis, hydration, fatigue, emotion, digestion, et cetera, it was decided to confine attention to the chief factors in the practical application of sugar tolerance tests which might influence their specificity. From the work of previous investigators and our own experience it appeared that the chief factors are: (1) the time taken to make the test, (2) the antecedent diet or food habits of the subject and (3) the rate of absorption from the intestine of ingested glucose.

As usually performed, it takes two or more hours to make a sugar tolerance test, although it is clear that there are definite advantages in making the period of physiological experiments of this kind as brief as possible in order to exclude external and secondary influences on specificity such as emotional complica-

tions like anxiety and impatience, and physical discomforts like hunger, et cetera. For some years we have been giving a single 75 gram dose of glucose and limiting the test to two hours, counting a return of the blood sugar to within 20 per cent of the fasting value as the criterion for normals. In a very considerable amount of accumulated data no cases gave any more definite information at the end of three or four hours than at the end of two hours. In fact, the results of some cases seemed less obscure at the end of the shorter period. The patients preferred the shorter test in every instance, and the advantages of abbreviating the period have become still more apparent with the new procedure, which takes only an hour to complete.

Another and more important influence on the results of sugar tolerance tests is the antecedent diet or food habits of subjects. A number of authors have called attention to this factor, but none so vividly as Sweeney[16] in this country, some of whose data are shown in figure 1. His curves show the average responses of groups of healthy young adults to glucose tolerance tests after living on exclusive antecedent diets for 48 hours. The curves are particularly suggestive when it is remembered that all of the subjects gave normal curves when tests were made after antecedent mixed diets and that reversals of the curves occurred with the same subjects on changed diets. It will also be observed that although all of the subjects were known normals, those who took nothing but water and those who ate exclusively fats or proteins gave misleading results, while only those normals who ate carbohydrates gave normal responses to the tests.

Sweeney postulated that these differences are due to variations in the stimulation of insulin and concluded:

> The dextrose tolerance test as usually employed has been shown to be materially influenced by different antecedent foods or diets. . . . . Some of the curves believed to be peculiar to different pathological conditions may result from the diets.

As opposed to Sweeney's results with the older test, the theoretical basis of the new test implies that antecedent diets or food habits do not influence the results. We therefore tested a number of subjects under the conditions of their accus-

tomed food habits in accordance with Nielsen's[12] conclusion that there should be no alteration of a subject's food habits prior to the test. It was also thought that information secured in this way would conform with the general run of actual conditions in clinical and insurance practise better than information obtained under the less usual conditions of depriving subjects of their accustomed food and restricting them to unaccustomed exclusive diets.

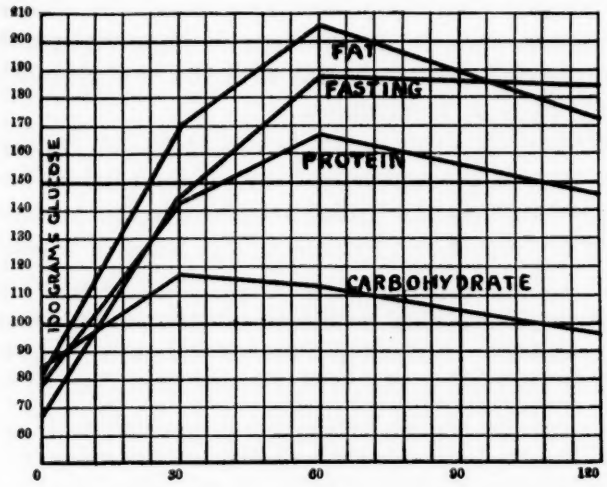

FIG. 1. Data of Sweeney showing effects of 48 hour exclusive antecedent diets on dextrose tolerance tests. Averages of four normal young adults on protein, five on fat, eight on carbohydrates and five on nothing but water. Only those on carbohydrates gave a normal response. *In all of the figures, time in minutes is plotted on the abscissa and milligrams of sugar per 100 cc. of blood are plotted on the ordinate.*

Two groups were made of sixty-five males and females, from eighteen to sixty-four years of age. Of these, some were known to be living on more or less exclusive diets. The first group consisted of six diabetics and forty-five non-diabetics. All of the members of this group were tested, not after the usual overnight fast, but at random times and regardless of what or when they ate. The second group consisted of fifteen diabetics, all of whom were tested, not at random times, but only after an overnight fast.

In both groups all tests gave perfectly typical results regardless of food conditions. Hence, antecedent food habits or diets do not affect the results of the new procedure.

Of all of the factors which influence the results of sugar tolerance tests the absorption of the ingested glucose from the intestine is by far the most troublesome. Woodyatt, Sansum and Wilder[18] held that the irregularities inherent in absorption vitiated the results of alimentary sugar tolerance tests and proposed an intravenous procedure designed to avoid them.

Following their work, Beeler, Bryan, Cathcart, and Fitz,[2] after reviewing earlier experimental work, proposed "an improved alimentary glucose tolerance test" in which the usual procedure was modified by emptying the stomach with a tube an hour after the ingestion of glucose. By measuring the difference between the ingested glucose and the amount thus recovered from the stomach they aimed to ascertain the amount of glucose actually absorbed, and to interpret their results on that basis. They summarized their interesting experiments, which also cover some of the unavoidable complications of absorption, as follows:

> Clinical observations emphasize certain obvious defects in the alimentary glucose tolerance test. . . . . They have not received sufficient attention. The absorption of glucose from the intestinal tract is a complicated process. The rate of absorption varies with each individual within wide limits. . . . . Glucose, in between 10 and 20 per cent solution, disappeared most rapidly from the stomach.

Their conclusions are of special interest when related to the concentrations of glucose employed in prevailing procedures which vary more on this than they do on any other point, although this is often obscured by insistence on other details, like the time allowed for drinking the glucose solution and its palatability.

It is typical of present practise to give 100 grams of glucose in 250 cc. of water. Some try to avoid the hypertonic effects of the 40 per cent solution by giving less glucose and others by giving more water. On this point a recent study of Magers[10] confirms the findings of Fitz and his coworkers because no consistent differences appeared in the blood sugar curves of twelve subjects who were given 50 grams of glucose in 15 per cent solution, orally and

intraduodenally. In line with the experimental evidence that concentrations of glucose of about 15 per cent are least likely to impair results, the new test employs two 50 gram doses of glucose in 15 per cent solution.

THE PARADOXICAL LAW OF DEXTROSE

On the basis of animal experiments Allen[1] enunciated his paradoxical law as follows:

> The paradoxical law of dextrose distinguishes sharply between diabetic and every type of non-diabetic animals. . . . . Limits of tolerance in non-diabetic animals are all apparent, not real. The limits of tolerance in diabetic animals are real, not apparent. Just the opposite of the paradoxical law. . . . . Whereas in normal individuals the more sugar given the more is utilized, the reverse is true in diabetes.

Allen was the first to suggest that his law might have diagnostic value, and Hamman and Hirschman,[7] studied the effect on the blood sugar of the repeated ingestion of glucose. They included protocols which show the effects of the blood sugar of diabetics and non-diabetics when a similar dose of glucose is ingested three hours after a first dose. This effect has also been studied abroad under the name of the Staub[15]-Traugott[17] phenomenon and other names, and the trend of these studies is illustrated in the recent text of Grafe,[5] by blood sugar curves showing the effects of repeating a 20 gram dose of glucose one and a half hours after the original dose.

In an effort to differentiate between the glycosurias of diabetes and hyperthyroidism Rabinowitch[13] made a particularly interesting application of Allen's paradoxical law by giving diets of constant composition with reference to protein and fat, but increasing the amounts of glucose. According to his protocols 10 grams of glucose were given every hour for ten hours one day, 20 grams similarly the next day, and 30 grams on ten succeeding days.

It thus appears that under varied experimental and clinical conditions many observers have independently established the fact that normal human beings react to repeated doses of glucose with either hypoglycemia or little or no change of glycemia, while diabetics react with definite hyperglycemia. The underlying

physiology has been satisfactorily explained by MacLean and de Wesselow,[9] and very well stated by Foster[4] in summarizing his own experiments:

> We interpret this fact as meaning that the first dose of glucose stimulates the insulin-glycogen mechanism to such activity that the normal organism is then able to deal with any amount of glucose without becoming hyperglycemic.

To this it may be added that diabetics react with distinct hyperglycemia because the insulin-glycogen mechanism fails.

We have, therefore, tried out the possibilities of the paradoxical law by varying the experimental conditions under which repeated tests were made on the same subjects, and have elsewhere presented a summary of these tests. They gave no evidence of being in any way affected by antecedent diets or irregularities of absorption, and very definitely indicated the practicability of adapting the paradoxical law to routine clinical use. They also enabled us to define optimum conditions, and formulate a simple and convenient procedure which has since been applied to more than four hundred individuals under usual clinical conditions.

THE ONE-HOUR TWO-DOSE TEST

Procedure

We perform the test by dissolving 100 grams of glucose in about 650 cc. of water. This solution is then flavored with lemon and divided into two equal doses containing 50 grams of glucose in about a 15 per cent solution, which are served cold and are easy to drink within a minute. We also keep at hand three containers with preservative against glucolysis[14,8] for the blood specimens and three containers for the urine specimens, making it a point when collecting these to have subjects empty the bladder as completely as possible. The following steps are taken after a fast, preferably overnight:

(1) Collect blood and urine (A) samples and give the first dose of glucose, allowing one to two minutes for its ingestion.

(2) Thirty minutes after ingestion of the glucose, collect blood (B) sample and give the second dose of glucose, allowing one to two minutes for its ingestion.

(3) Thirty minutes after the ingestion of the second dose of glucose, collect blood and urine (C) samples. The third urine container is given the subject for a sample of the urine next voided whenever a (post) sample is desired.

When the results of the foregoing procedure are plotted, the interpretation of the first part of the curve, that is, the part which

includes the original and the 30 minute samples, is exactly the same as the interpretation of the same part of the curve of the older procedure, and the same deductions are accordingly drawn from the blood and urine sugar values. The interpretation of the second part of the curve, the part which includes the 30 and 60 minute values, is not at all like that of the older test, and the following cases will serve to illustrate the criteria we use for interpreting the results of one-hour two-dose tests. Figure 2 shows the average responses of ten normal males and ten normal females,

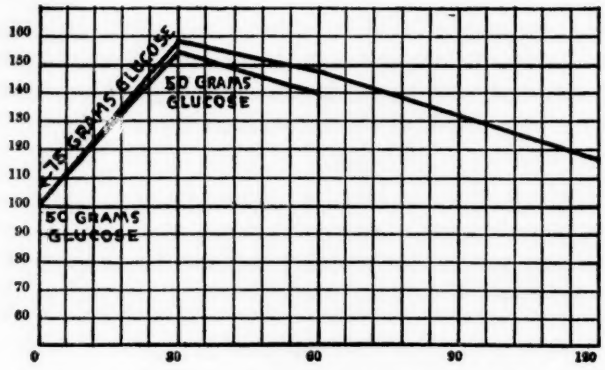

FIG. 2. Responses of ten normal males and ten normal females (ages 19–48) to one 75 gram and two 50 gram doses of glucose. Note the decline in blood sugar after the second dose of glucose; no sugar in urine.

19 to 48 years of age, to one 75 gram and two 50 gram doses of glucose.

Starting at 100 milligrams it will be seen that the first parts of both curves are practically the same. The rise in blood sugar following 75 grams is usually a little higher than that following the 50 gram dose of glucose. This tendency is, however, by no means invariable because many cases show the same or a higher rise in the blood sugar after 50 grams than they do after 75 or even 100 grams of glucose. Such instances are caused by more rapid and uniform absorption of the smaller amounts or concentrations of glucose, and an illustration of such a reversal of the expected results is seen in fig. 7.

The second part of the one-hour two-dose curve, that is, 30 and

60 minute values, indicates that normals respond to a second dose of glucose with a greater fall in blood sugar than that occurring during the same period after only a single dose of glucose. It is also characteristic of normals that there is no sugar in the urine. *The typical criteria of normal responses to the one-hour two-dose test are, therefore: (1) a fasting blood sugar within the normal limits of the particular blood sugar method employed; (2) a rise in blood sugar which does not exceed 75 mgm. in the 30 minute sample; (3) the blood sugar in the 60 minute sample is less, the same, or does not exceed the 30 minute sample by more than 5 mgm., and (4) all urine samples are negative to Benedict's test.*

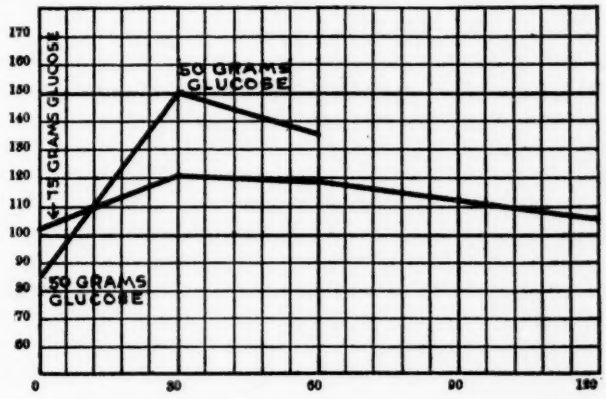

FIG. 3. CASE 1. Illustrates a typical flat curve of older test and typical normal result of new test.

The extent to which certain results detract from the clinical value of sugar tolerance tests was indicated by the quotations from authorities in the introductory part of this paper. Some of these questionable results occur in the form of curves having delayed peaks or curves with plateaux or curves which tend to flatness. We have had a number of these cases. Although the responses to the older test were doubtful or misleading, all of them gave perfectly typical responses to the one-hour two-dose test. An obvious explanation of these discrepancies is offered by the differences observed after introducing glucose into the duodenum at various rates which Hale-White and Payne[6] have interpreted

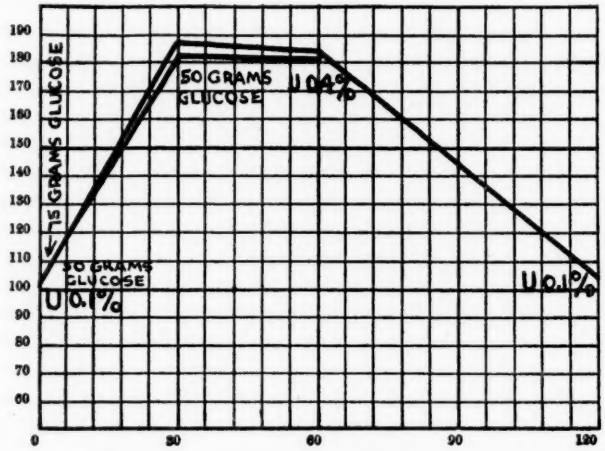

FIG. 4. CASE 2. Has sugar in urine occasionally. Illustrates so-called plateau curve of old test. By the new test, the typical normal shape of the curve, the higher than normal level of the blood sugar, and the concentration of sugar in the final urine indicate a mild alimentary glycosuria. (Case of Dr. Wm. Tilton.)

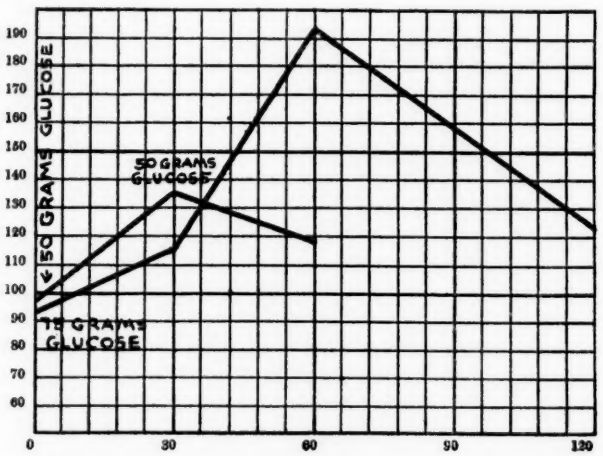

FIG. 5. CASE 3. Illustrates delayed peak curve of older test in a normal subject, probably due to delayed absorption of glucose. No sugar in any of the urine samples but blood sugar values of older test make the diagnosis doubtful. The result of the new test is typically normal.

as meaning that the emptying rate of the stomach influences the glycemic period. Cases illustrating flatness, a plateau and a delayed peak, in the curves given by the older test, but perfectly typical responses to the new test are seen in figs. 3, 4 and 5.

Unlike the preceding curves, those in fig. 6 represent the characteristic responses of a patient with mild diabetes. The responses of another mild diabetic in fig. 7 show a perfectly typical result of the new but an atypical result of the older test. In com-

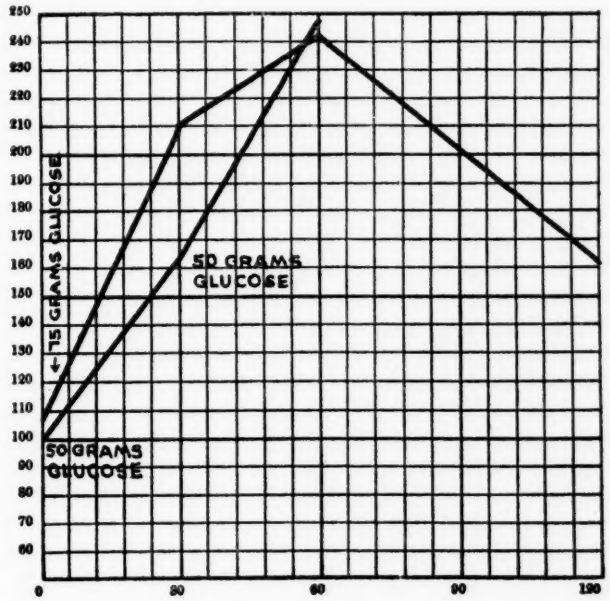

FIG. 6. CASE 4. The steep climb of the blood sugar curve after the second dose of glucose is typical of diabetes. (Case of Dr. W. R. Tilton.)

paring the responses of these diabetics to the older tests at the end of the two hour periods it is evident that the hyperglycemia in case 5 (fig. 7) is increasing when case 4 (fig. 6) is decreasing. In case 4 it is also evident that the rise in blood sugar after 75 grams of glucose is greater than that after 50 grams, as it logically should be, while in case 5 the blood sugar rises higher after the lesser amount of glucose, contrary to theoretical expectations. This case also illustrates how some diabetics, as well as normal

persons, respond to the older test with atypical results due to irregular absorption while the results of the new test are perfectly typical.

In the one-hour two-dose tests of both mild diabetics it is apparent that the first parts of the curves are similar in trend to those of the older tests but that the latter parts which show the effects of the second doses of glucose are distinctly different. Here the steep climb of the blood sugar characterizes diabetes, and sharply distinguishes it from all non-diabetic conditions in accordance

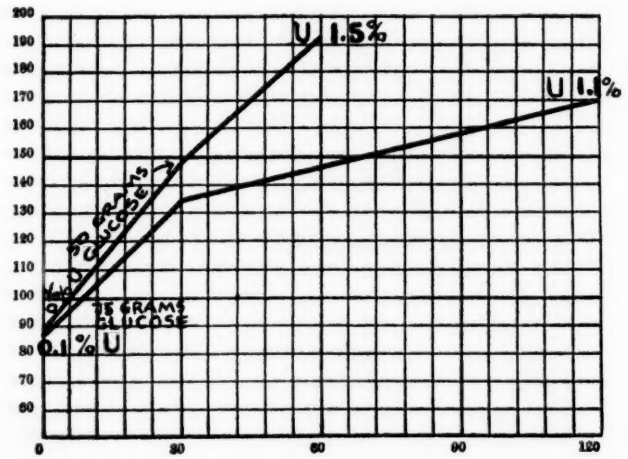

FIG. 7. CASE 5. Mild diabetes showing atypical result of older test due to irregular absorption and typical result of the new test. (Case of Dr. E. S. Dillon.)

with the paradoxical law. In both cases it will also be noted that there is no sugar in the urines from the fasting patients, but sizeable concentrations in the samples voided after ingestion of glucose.

In more severe or advanced diabetes the responses to the one-hour two-dose test are in a general way the same as the responses of mild diabetes, only the sugar values run higher in all the blood and urine samples of the severer cases. The tendency for the difference between milder and more severe diabetes to be quantitative is apparent in the curves of fig. 8 which shows the results

of old and new tests in a case of more severe diabetes. From these illustrations it is evident that *the criteria for determining diabetes in the one-hour two-dose test are a more or less steep rise of not less than 10 mgm. of blood sugar in the 60 minute sample following the*

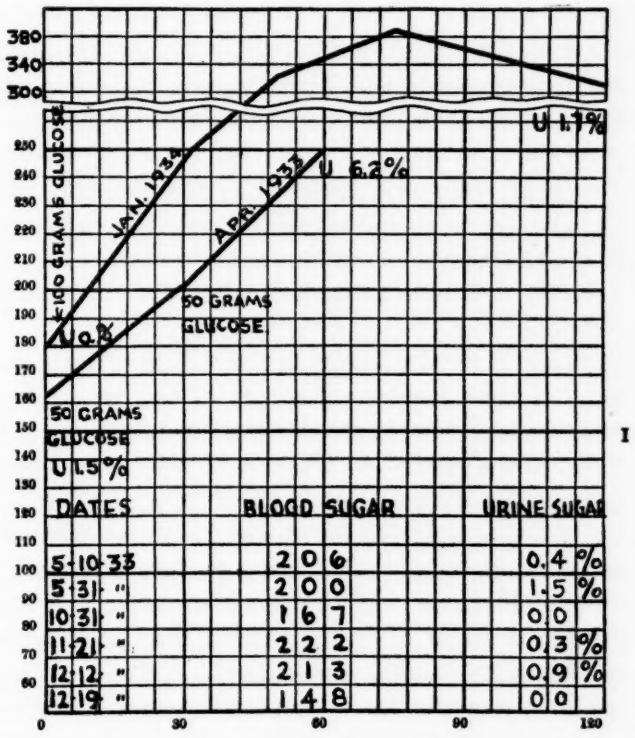

FIG. 8. CASE 6. Illustrates typical results of both tests in more severe diabetes, i.e., same criteria as mild diabetes but higher sugar values related to the severity of the disease. Case also illustrates tendency to high and changing renal threshold for glucose. (Case of Dr. H. O. Mosenthal.)

second dose of glucose and the relation of blood and urine sugar values to the severity of the disease.

In some cases of diabetes the renal threshold for glucose changes from time to time as it does in non-diabetic derangements of carbohydrate metabolism. Case 6 shown in fig. 8 also illustrates such an instance. In some of the cases the blood sugar values

may run very high when the urine contains little or no sugar. These tendencies are not characteristic of any particular condition but more likely associated with complications or with derangements of carbohydrate metabolism which may be secondary to some clinical condition other than diabetes.

A wrong diagnosis of diabetes is made more often in cases of renal glycosuria and pentosuria, especially the latter, than in other persistent glycosurias. Furthermore, it is not at all exceptional to find cases of pentosuria also incorrectly diagnosed as renal glycosuria. As both of these conditions give similar responses to sugar tolerance tests, this mistake can hardly be avoided unless the precaution is taken to identify the sugar found in the urine. In order to avoid unnecessary alarm and treatment it is particularly desirable to do this when the concentrations of sugar are small.

The diagnosis of renal glycosuria rests upon criteria which are still subjects of considerable difference of opinion and practise. Perhaps the strictest criteria are those of Joslin and his associates, and fig. 9 shows the results of old and new tests on one of their cases of renal glycosuria, (No. 2279 of Marble's[11] report). It should be noted that different blood sugar technics were employed in making these curves and that the new test was made several years after the older one. In the one hour curve it is of interest to find the blood sugar after the second dose of glucose in the borderland between normal and diabetic values. While other cases of renal glycosuria have shown the same tendency, more cases show blood sugars like normals. The cases of pentosuria behave in the same way, and it is, of course, characteristic for all urine samples to show sugar in both renal glycosuria and pentosuria. *The criteria of the new test for renal glycosuria are, therefore: blood sugars which follow the normal course, or in any event never reach the diabetic level, and sugar in both urine specimens.*

In addition to the persistent glycosurias, there are intermittent glycosurias which may or may not be of diabetic origin. In these cases the sugar tolerance test is the only means at our disposal for distinguishing between diabetes and alimentary glycosuria. In the cases of alimentary glycosuria the fasting blood sugar is within

normal limits like some of the diabetic cases. Following the first dose of glucose the 30 minute blood sugar in some of these cases may remain within normal limits, but like the diabetics it more usually exceeds these, often very considerably. After the second dose of glucose, however, a very distinct difference appears between the alimentary glycosuria and cases of diabetes. The blood sugar distinguishes sharply between them because it rises characteristically in diabetes and falls in alimentary glycosuria as in normals. In both conditions the final urine samples show more

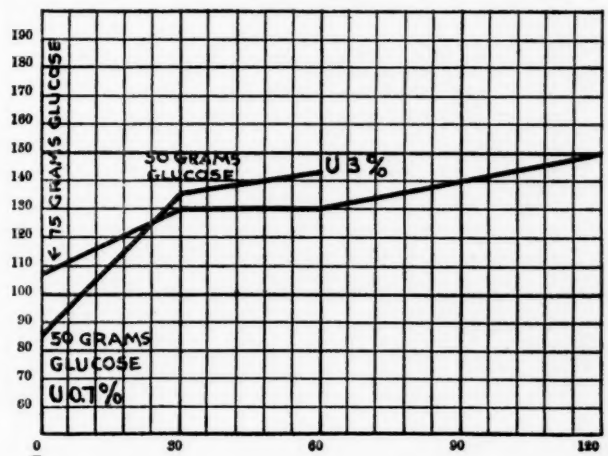

FIG. 9. CASE 7. Renal glycosuria. The blood sugar does not reach the diabetic level after a second dose of glucose. (Case of Drs. Joslin and Marble.)

or less sugar, depending upon the threshold of the individual. Figure 10, when compared with the diabetic curves in figures 6, 7 and 8, shows the characteristic distinction between alimentary glycosuria and diabetes. It should also be compared with the milder case of alimentary glycosuria with plateau curve of the older test seen in fig. 4. *The criteria of alimentary glycosuria are, therefore: a sugar-free urine after fasting with sugar in the final urine and blood sugars that follow the normal curve even when the level is higher than normal.*

While the foregoing conditions represent types of glycosuria in

which the carbohydrate metabolism is of special clinical interest, there is a variety of other conditions which secondarily or accidentally affect carbohydrate metabolism. Some of them are associated with glycosuria, generally intermittent in character; others are not associated with glycosuria. In some of the cases the disturbance of carbohydrate metabolism is temporary or trivial, as in colds and other infections or toxemias, menstruation, et cetera, and it is, of course, advisable not to make sugar tolerance tests at

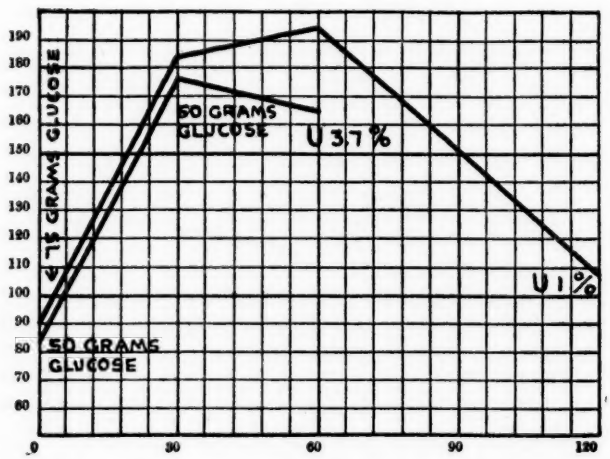

FIG. 10. CASE 8. In alimentary glycosuria there is no sugar in the urine when patient is fasting but definite amounts after ingestion of glucose. The new test sharply distinguishes between this condition and diabetes by the fall in blood sugar after the second dose of glucose instead of the rise characteristic of diabetes. Compare with fig. 4 showing a similar but milder case. (Case of Dr. P. V. Reinartz.)

such times. In other cases the glycosuria depends on more fundamental disturbances of carbohydrate metabolism which must be differentiated from diabetes, such as those associated with endocrine and psychic conditions. Cases illustrating equivocal results of the older test in thyroid and emotional conditions but unequivocal results of the new test are shown in figures 11 and 12.

Up to the present time not a single instance has been encountered in which the new test disagreed with the older when the

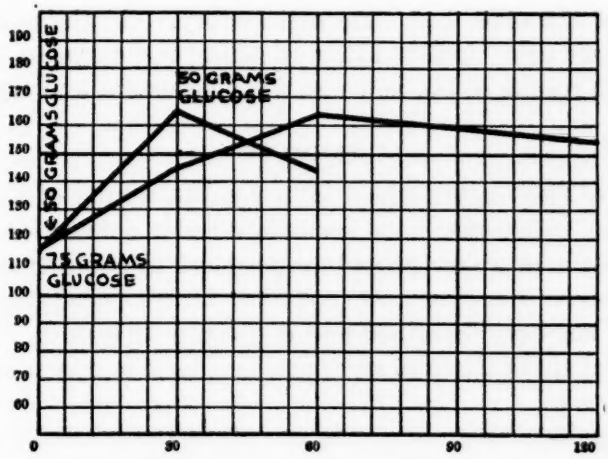

FIG. 11. CASE 9. Nervous and irregular menses. Under treatment for thyroid trouble. Sugar in urine occasionally, the new test definitely excludes a diabetic tendency but the older test does not. (Case of Dr. M. K. Smith.)

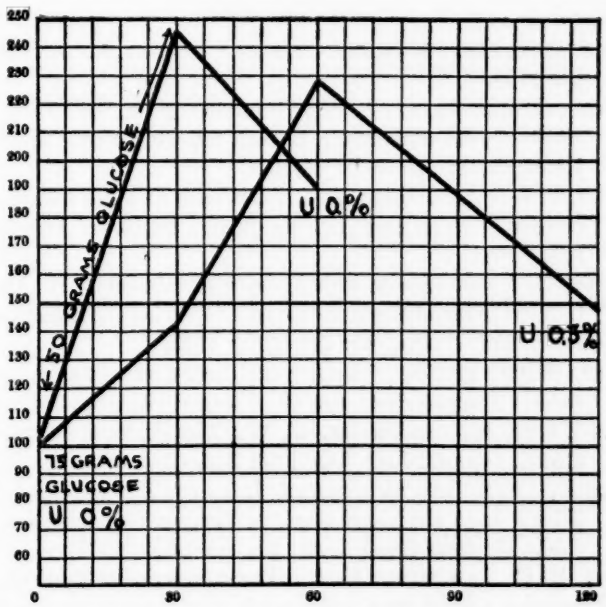

FIG. 12. CASE 10. Illustrates psychic effects on sugar tolerance tests. In this case, emotional disturbance related to secret marriage and early pregnancy. The older test indicates diabetes but the new test does not. (Case of Dr. W. R. Tilton.)

results of the older test were satisfactory. On the other hand, many cases gave doubtful or misleading responses to the older test when results of the new test were consistent and specific.

SUMMARY

Some of the factors which influence the results of alimentary glucose tolerance tests have been discussed in connection with the development of a new procedure designed to avoid them.

A one-hour two-dose test based on Allen's paradoxical law of dextrose has been described and its diagnostic criteria illustrated by clinical applications of older and new tests to more usual types of glycosuria.

Besides its greater convenience, all of the available evidence indicates that results of the new test are more specific and reliable.

We express our appreciation of the careful technical work of Miss Cecile Blacker in executing these studies.

REFERENCES

(1) ALLEN, F. M.: Glycosuria and diabetes. Boston: W. M. Leonard, 1913, pp. 1179.

(2) BEELER, CAROL; BRYAN, A. W.; CATHCART, E. P., AND FITZ, REGINALD: An improved alimentary glucose tolerance test. Jour. Metabolic Research, **1:** 549–563. 1922.

(3) EXTON, WM. G., AND ROSE, A. R.: Diabetes as a life insurance selection problem. Proc. Assn. Life Ins. Med. Directors of America, **18:** 252–273. 1931.

(4) FOSTER, G. L.: Studies on carbohydrate metabolism. II. An interpretation of the blood sugar phenomena following the ingestion of glucose. Jour. Biol. Chem., **55:** 303–314. 1923.

(5) GRAFE, ERICH: Metabolic diseases and their treatment, Trans. by M. G. Boise. Philadelphia: Lea and Febiger, 1933, pp. 551.

(6) HALE-WHITE, R., AND PAYNE, W. W.: The dextrose tolerance curve in health. Quart. Jour. Med., **19:** 393–410. 1926.

(7) HAMMAN, LOUIS, AND HIRSCHMAN, I. I.: Studies on blood sugar. IV. Effects upon the blood sugar of the repeated ingestion of glucose. Johns Hopkins Hosp. Bull., **30:** 306–308. 1919.

(8) LEWIS, R. C., AND MILLS, G. C.: The comparative value of monochlorbenzene and thymol when used with fluoride as preservatives of blood for chemical analysis. Am. Jour. Clin. Path., **3:** 17–28. 1933.

(9) MacLean, H., and de Wesselow, O. L. V.: The estimation of sugar tolerance. Quart. Jour. Med., **14:** 103–119. 1921.

(10) Magers, E. J.: A comparison of blood sugar curves when glucose is given orally and intraduodenally. Jour. Lab. and Clin. Med., **19:** 608–611. 1934.

(11) Marble, Alexander: Renal glycosuria. Am. Jour. Med. Sci., **183:** 811–827. 1932.

(12) Nielsen, O. J.: On oscillations of blood-sugar values within brief periods, and the blood-sugar curve on uniform ingestion of glucose. Biochem. Jour., **22:** 1490–1498. 1928.

(13) Rabinowitch, I. M.: The glycosuria of hyperthyroidism and its clinical significance. Ann. Int. Med., **4:** 881–896. 1931.

(14) Rose, A. R., and Schattner, Fred: Glucolysis in blood samples. Am. Jour. Physiol., **84:** 103–109. 1928.

(15) Staub, H.: Bahnung im intermediären Zuckerstoffwechsel. Biochem. Zeitschr., **118:** 93–102. 1921.

(16) Sweeney, J. S.: Dietary factors that influence the dextrose tolerance test. Arch. Int. Med., **40:** 818–830. 1927.

(17) Traugott, Karl: Über das Verhalten des Blutzuckerspiegels bei wiederholter und verschiedener Art Enteraler Zuckerzufuhr und dessen Bedeutung für die Leberfunktion. Klin. Woch., **1:** 892–894. 1922.

(18) Woodyatt, R. T., Sansum, W. D., and Wilder, R. M.: Prolonged and accurately timed intravenous injections of sugar. Jour. Am. Med. Assn., **65:** 2067–2070. 1915.

SOME MEDICOLEGAL ASPECTS OF ISO-AGGLUTININS*

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Although the discovery of blood groups, by Landsteiner in 1900, has proved its value in medicolegal cases abroad, the legal profession of this country has failed properly to recognize its possibilities. In Europe the reliability of blood grouping has been established by more than 10,000 paternity cases alone, yet the literature of this country fails to record a single case in which evidence obtained by blood grouping was admitted as competent testimony in an American court, and in which a judge handed down an opinion concerning the value of such evidence.

The importance of the universal recognition of the laws governing the heredity of the blood groups can be realized when one considers the results of more than 5000 cases of disputed paternity, reported by Schiff[7] of Berlin in 1929. Had all of the accused men been innocent, only 16 per cent could possibly have proved non-paternity by the methods in use at that time. Actually, the innocence of half that number, or 8 per cent, was definitely established, from which it follows that one-half the total accused group in reality was not guilty. Therefore, assuming the same distribution of guilt and innocence in European and American groups, we must conclude that one-half the defendants in fornication and bastardy cases are falsely accused of at least half of the charge. Also since accusations of bastardy are usually impossible to disprove by the methods now in use in this county, convictions are almost certain to follow, or if cases are settled out of court in order to avoid scandal, a legalized form of blackmail is possible. Furthermore, the knowledge of blood groups has solved the problem of mixed children, and also furnished valuable clues in criminology.

* Read before the Thirteenth Annual Convention of the American Society of Clinical Pathologists, Cleveland, Ohio, June 8 to 11, 1934.

THEORY

In 1910 von Dungern and Hirschfeld[2] concluded that the blood groups were inherited as two independent pairs of Mendelian factors. They believed that the iso-agglutinogens A and B were dominant to the corresponding iso-agglutinins a and b. On the basis of this theory they correctly assumed that the factors A and B could not appear in a child unless they were present in at least one of the parents. This theory of independent pairs of factors is untenable because it does not explain the percentages of the blood groups of children born of parents of known blood groups, and, furthermore, erroneously admits the

TABLE 1

GROUPS OF PARENTS	GROUPS OF CHILDREN POSSIBLE	GROUPS OF CHILDREN NOT POSSIBLE
O × O	O	A, B, AB
O × A	O, A	B, AB
O × B	O, B	A, AB
A × A	O, A	B, AB
A × B	O, A, B, AB	
B × B	O, B	A, AB
AB × O	A, B	O, AB
AB × A	A, B, AB	O
AB × B	A, B, AB	O
AB × AB	A, B, AB	O

possibility of group O offspring from an AB parent. Bernstein[1] proposed a theory which agrees with the actual facts of heredity, namely that there are three multiple allelomorphs A, B, and O. Since A and B act as dominants, and O as recessive, table 1 illustrates the possibilities of inheritance of blood groups.

Although some investigators have occasionally found exceptions to the above, these can be attributed to technical errors or to illegitimacy. It seems that the advice of Ottenberg[6] has not always been heeded, namely, to acquaint the mother with the purpose of the test so that she can object if she has reason to be in doubt as to the actual fathers of her children. By following Ottenberg's plan I have been able to determine the blood groups

of 246 "certified" parents and their 259 children, and it will be seen that no exceptions to the Bernstein theory are found (table 2).

If, in addition to the above hereditary factors, A and B, the recently discovered agglutinogens M and N described by Landsteiner and Levine[4] are also considered, the medicolegal value of blood grouping is about doubled.

TABLE 2

MATING	NUMBER OF FAMILIES	CHILDREN IN GROUP			
		O	A	B	AB
O × O	18	33			
O × A	46	36	42		
O × B	9	19		6	
A × A	28	15	47		
A × B	11	9	8	8	4
B × B	1	1			
AB × O	4		5	5	
AB × A	6		10	1	10

CASE REPORTS

The following cases from my own experience are cited because they illustrate some important uses of blood grouping.

Case 1. J. P. died shortly after being struck by an automobile. No witnesses were immediately available. The discovery, about a week later, of dried blood on rocks some fifty feet from the site of the accident led to the suspicion that the man had been attacked near the rocks and then dragged into the road. Blood from the rocks and from the clothing worn by the victim on the day of his death was found to be Group B. Witnesses appeared later who testified that they had removed the victim to the place where the blood was found *after* the accident. Although no new facts were obtained by blood grouping in this case, it is cited because it illustrates the feasibility of correctly grouping dried blood which had been exposed to the elements.

Case 2. A middle aged man, was accused of sodomy by an eight year old girl. This man had been tried for similar offenses at least five times and had served two sentences. His technic had always been the same: to lure his victim to a secluded spot, give her a nickel, assault her, and take back his nickel. Spots on

the child's bloomers contained spermatozoa and the agglutinogens were of Group A. A Wassermann test was ordered on the man for the protection of the child, thus affording the opportunity of determining that his blood group was A. Though insufficient in itself, this finding, in conjunction with the other evidence presented at the trial, was sufficient to bring a verdict of guilty and a prison sentence.

Case 3. J. N., a colored man of Greensburg, Pennsylvania, was arrested on a charge of raping a seven year old white girl. The girl was positive in her identification Spots of semen on her clothing were identified, and the agglutinogens in this case found to be group B. A specimen of blood from the accused man proved to be of group A, and on this evidence alone the man was released. Subsequent developments justified this decision.

FIG. 1. THE ACCUSED

*Case 4.** This case is presented in some detail, since it appears to be the first of its kind in this country and thus established a much needed precedent.

On April 23, 1931, there came to the laboratory of the Uniontown Hospital a young man (fig. 1) of Italian parentage with an unmarried girl seventeen years of age and her infant son, four months old. The young man's uncle had persuaded his nephew and the girl to have their bloods tested to determine the

* June Sessions 1931, Fayette County, No. 140.

guilt or innocence of the man, whom the girl had accused of being the father of her child. That the test has limited application to the proof of non-paternity was explained, and before proceeding further, the interested parties signed a statement that the test was being made at their request, and that if either party was not satisfied with the result, they would all agree to be tested in some other laboratory. The results were as follows: young man, Group O; mother, Group A; baby, Group B. Additional data are shown in table 3.

These tests confirm the blood groups of the man and woman, and show that the baby's serum had not as yet developed isoagglutinins.

Although the District Attorney and the mother were informed that the accused could not have been the father of her child, the case came to trial on June 5, 1931.* The woman testified that the defendant had had sexual intercourse with her at various times between February and September 1930, on "a pile of

TABLE 3

ADDITIONAL DATA ON CASE 4

SERUM USED	CELLS	AGGLUTINATION
Baby	Mother	−
Baby	Man	−
Baby	Known A	−
Baby	Known B	−
Mother	Baby	+
Mother	Man	−
Man	Baby	+
Man	Woman	+

logs near the horse field." The man admitted a casual acquaintance but denied having had sexual intercourse with her.

The District Attorney's objection to the introduction of the medical testimony included the following:

> The determination of parentage is still in an experimental stage and is purely theoretical; that it has never yet been recognized by the medical profession as proof of parentage without too great a degree of error to be acceptable in evidence; that the tests were taken of the mother and child upon misrepresentation to her that it would determine the parentage of the child; and that it is an invasion of the privacy of the mother and the child. Further, that evidence of this kind has never yet been admitted as evidence in a criminal proceeding in the State of Pennsylvania, and that it would be a dangerous precedent to set.

The attorney's objections were overruled, and after much argument, the writer's testimony was admitted as competent.

* Mr. Wade Newell, the attorney defending the accused man, deserves credit for his handling of this pioneer case.

In cross examination the District Attorney entered into a vigorous attack on the "so-called blood test," and was greatly disconcerted by some of the answers made to his questions:

q. "And is it not also true that a good many pathologists are of the opinion that the parentage of a child cannot be determined by a blood test?"
a. "They all agree on that subject."
q. "They all agree?"
a. "All agree."
q. "That they can be determined?"
a. "That they cannot."
q. "That they cannot be?"
a. "That is correct."
q. "Then of what value is the blood test to determine parentage?"
a. "None."

It was not until later that he realized that the purpose of the testimony was not to prove paternity, but to prove non-paternity.

Many quotations from the Journal of the American Medical Association were placed in evidence by the District Attorney in an attempt to prove that the medical profession had no faith in the value of blood grouping for this type of case. His trump card was a quotation from volume 94, page 1089, April 5, 1930, which is an inquiry addressed to the editor:

I would like some information concerning the present status for medical purposes of all paternity tests by blood grouping; I especially desire to know whether one or both alleged parents can be definitely ruled out if the child in question does not follow in the blood group of one or the other?

ANSWER: The answer to the question as stated is, No. Neither alleged parent can be ruled out simply because the child does not fall in the group of one or the other. For example, parents of groups A and B may have children of all four groups. The child of group O, for instance, could have one parent of group A and the other of group B. This would also be true of a child of group AB. Again a child of group O could have both parents in group A or group B. A child of group B could have one parent in group A, the other in AB. In none of these cases does the child belong to the group of either parent.

Although the above quotation had no direct bearing on this case, the jury failed to understand the significance of the evidence offered, and apparently accepted it as proof of the incompetence of the medical testimony.

In order to prove that blood grouping was in the "experimental" stage and purely theoretical the District Attorney cited the conflicting theories of von Dungern and Hirschfeld with that of Bernstein, and the testimony failed to impress him or the jury that theory and fact were entirely different propositions.

When the judge finally admitted the medical testimony, it was clearly stated that a mother of group A could not have a group B child from a group O man, and that the defendant was not the father.

After the conclusion of the medical testimony the District Attorney exhibited the infant to the members of the jury, calling their attention to the facial resemblance and other points of similarity to the defendant, and said,

The old fashioned evidence produced by eye-witnesses and which has been good enough for these courts from the time they started should not be dashed aside for this new fangled theory yet in its experimental stage.

During the final argument to the jury the Assistant District Attorney made the following statement, with appropriate gestures:

Possible groups of children; if he were certain at the time he made this chart out (referring to table 1), why did he put possible groups of children. In other words, here is what it is: If the parents are O and O, the possible group of the children would be O; if they were O and A, the possible group would be O and A—a possibility. Where is there any certainty about that? Does that exclude this young man? Possibly! The first time I heard about this grouping business was out in Chicago where two children got mixed in the hospital. Well, they grouped and grouped, and as far as I know they are grouping yet, and they are farther away from it than when they started. That is how old and tried this grouping of blood is to determine non-parentage. The mothers finally decided: This is my baby, and the other mother said, This is my baby, and they all went home happy and forgot all about this grouping business.

For the sake of appearances the jury "deliberated" a total of ten minutes and returned a verdict of guilty. When later questioned, one of the more intelligent jurors stated the reason for the verdict as follows:

Of course, we didn't pay any attention to your testimony, and, furthermore, the baby is the image of his grandfather (the father (fig. 2) of the defendant, who appeared as a witness).

After the verdict the attorney for the defense made a motion for a new trial. The opinion of the Judge is given special consideration because it is the result of several months of study by Judge John Morrow[5] of Uniontown, who presided at the trial, and his recorded statement, November 7, 1931, furnishes the first legal precedent in this country for the recognition of the importance of blood grouping to exclude paternity. After analyzing the evidence and personally delving into the literature, he concluded that the verdict was against the evidence and granted a new trial. However, this trial has never taken place.

Probably the most valid objection to the acceptance of the medical testimony was the fact that we still tolerate three different methods of designating the blood groups. I agree with the District Attorney that this state of affairs gives the impression of lack of agreement among the members of the medical pro-

fession. Certainly there would be less confusion if the two contradictory methods of designating blood groups by numbers were replaced by the new Landsteiner classification. This classification has international scope and further is the only system which clearly indicates the genetic relationship of the

FIG. 2. THE FATHER OF THE ACCUSED

groups. I disagree with Kennedy,[3] that "whichever classification is most prevalent would, to my mind, be the logical one for adoption."

Almost three years after the trial, unexpected characteristics began to develop in the boy whose parentage had been so clearly decided by an intelligent jury.

With great difficulty, contact with the family was made and permission to take a picture of the mother and baby was given. This picture suggests heredity which is not purely Caucasian and the mother's honesty in designating the woodpile as the locale of her amorous adventures is made painfully evident by the photograph of the child. (Fig. 3.)

FIG. 3. THE MOTHER AND CHILD
This photograph was taken three years after the trial

CONCLUSIONS

1. The importance of blood grouping to determine non-paternity is evident when we consider that about half of the men accused in paternity cases are not the true fathers.

2. The laws governing the heredity of blood groups are well known and properly controlled observations of the blood group of the true parents and their children, fail to reveal exceptions to these laws. Personal observations of the blood groups of 240 "certified" parents and their 259 children are quoted.

3. The confusion in the lay mind arising from the existence of three methods of designating the blood groups, should be ended by having this society officially adopt the Landsteiner classification. This classification is urged, first because it gives

proper credit to the original discoverer of the blood groups; second because it is international in scope; third, it eliminates two contradictory methods of designating the groups by numbers; and, finally, the Landsteiner terminology indicates the genetic relationships of the blood groups.

4. Details of a court trial are given, showing the inability of the prosecuting attorneys to understand the scientific basis and value of blood grouping, and a final jurors' verdict based on the "good old fashioned evidence of eye witnesses and not upon this new fangled theory" is given.

5. I believe that no case of fornication and bastardy should appear in the courts of our country unless blood grouping, performed by properly qualified persons, fails to exclude paternity on the part of the accused. Also refusal of the mother to permit blood grouping should be given due consideration by the legal profession.

6. The opinion of Judge John Morrow furnishes a much needed precedent in recognizing the value of blood grouping.

REFERENCES

(1) Bernstein, Felix: Ergebnisse einer Biostatistischen zusammenfassenden Betrachtung über die erblichen Blutstrukturen des Menschen. Klin. Wchnschr., **3:** 1495–1497. 1924.

(2) von Dungern, E., and Hirschfeld, L.: Ueber Vererbung gruppenspezifischer Strukturen des Blutes. Zeitschr. f. Immunitätsforsch. u. exper. Ther., **6:** 284–292. 1910.

(3) Kennedy, J. A.: Blood group classifications used in hospitals in the United States and Canada. Jour. Am. Med. Assn., **92:** 610–615. 1929.

(4) Landsteiner, K., and Levine, P.: On individual differences in human blood. Jour. Exper. Med., **47:** 757–775. 1928.

(5) Morrow, S. J.: Opinion, No. 148 June sessions, 1931. Court of Quarter Sessions, Fayette County, Pennsylvania, November 7, 1931.

(6) Ottenberg, Reuben: Medicolegal application of human blood grouping. Jour. Am. Med. Assn., **79:** 2137–2139. 1922.

(7) Schiff, F.: The medico-legal significance of blood groups. Lancet, **2:** 921–922. 1929.

PREPARATION OF NEUROLOGIC MATERIAL FOR HISTOLOGIC STUDY*

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In order to arrive at a correct diagnosis of lesions of the nervous system, pathologists are faced with an overwhelming assortment of special staining methods which are tedious and complicated, and usually require special fixatives. There is much duplication of methods; this has the advantage of allowing the pathologist a wide choice, but has the disadvantage of leading to confusion. Even expert technicians find difficulty in employing many of these staining methods, and much simpler and less complicated and time consuming methods often serve the purpose equally well.

The suggestions on histologic methods which are offered here are not intended to take the place of text books on the subject of neurohistologic technic, or of methods used in neurologic research. They represent, merely, some of the common and comparatively simple methods used in the preparation of neurologic material for histologic study. Each pathologist, of course, develops in his own laboratory a few methods with which he becomes familiar and from which he obtains most information. His technicians also, as the result of frequent repetition, do better with these than with any other methods. The following methods, however, may be used to supplement those which are used routinely in most general histologic laboratories. The list is far from complete, but by the use of the methods given most of the elements in normal and abnormal tissues of the nervous system can be demonstrated.

It will be necessary on occasion to make a diagnosis on fresh neoplastic tissue removed at operation, so that the surgeon can

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decide what, if any, further operative procedure should be done. Staining fresh, frozen sections with polychrome methylene blue, as used by MacCarty and Broders for many years, is eminently satisfactory and is a method that has stood the test of time, although one of several more recent modifications may be adopted.

The proper fixation of tissue removed at operation is very important, and due regard must be paid to the nature of the tissue and to the staining methods which may be necessary to arrive at a correct diagnosis. It is occasionally impossible to foresee these conditions and, therefore, a few pieces as fresh as possible should be placed in Zenker's and in Cajal's solutions and the remainder in a 10 per cent solution of formol. When a neoplasm is present and the entire brain has been removed at necropsy, small portions should be fixed in similar solutions as recommended for tissues removed at operation and even though a neoplasm is not present, it is frequently advisable to remove small portions from various parts of the brain anyway and to place these in the Zenker and Cajal solutions before the brain is fixed in formol. In order to fix the brain in formol, the preferable method is to inject a 10 per cent solution of this mixture into one carotid and into one vertebral artery, or else to suspend the brain in the 10 per cent formol solution by a thread passed between the basilar artery and the pons and held so that the vertex does not rest on the bottom of the container. After the brain has been fixed for a few days, it is best to cut it by coronal sections about 1.5 cm. apart. This permits careful examination and easy reconstruction of the tissue, and also makes the sections suitable for photographing. Small portions can be removed at this time for microscopic preparation and study.

The universal fixative in all pathologic laboratories, of course, is a 10 per cent solution of formol, which has the advantage of preserving tissue almost indefinitely and of permitting the use of the majority of routine staining methods.

Stains for lipoids are sometimes necessary to demonstrate active degeneration of myelin sheaths in the brain, spinal cord, or in peripheral nerves (Method 1). This staining is done after

the tissue is fixed in formol solution and cut in frozen sections. Frozen sections are necessary in the Bielschowsky silver method to demonstrate axis-cylinders, but a modification of this stain can be used on formol-fixed tissue which has been embedded in paraffin. Contrary to the usual teaching, embedding of neurologic material in celloidin is not necessary except for some special and rarely used methods. I have long since ceased to use celloidin as a routine embedding medium and have substituted paraffin. After embedding in paraffin, the routine histologic stain, hematoxylin and eosin, is used. This is satisfactory to demonstrate inflammatory reactions and is the routine stain for all neoplasms of the central nervous system, although one or more special stains are used to confirm the diagnosis. Van Gieson's stain is useful to demonstrate connective tissue, but it is not so satisfactory for this purpose as the Perdrau silver method (Method 2). The impregnation method has been very satisfactory in this laboratory, but there are several other equally good silver impregnation methods for connective tissue (Laidlaw, Foot, and so forth). There are two rapid methods for demonstrating myelin sheaths after formol fixation and paraffin embedding (Loyez and Weil), but they are not as satisfactory as the original Weigert myelin sheath stain or Pal's modification of the original Weigert stain.

Changes occur in nerve cells as the result of trauma, inflammation, injury following toxins, deficiency in diet, poisons, and so forth, and, to see these changes, stains used to demonstrate Nissl's granules must be used. There are many such stains and all are modifications of Nissl's original methylene blue. Two of the most satisfactory of these are toluidine blue stain and thionin stain (Method 3). Many of the stains used to demonstrate Nissl's granules fade more or less rapidly, and the methods require overstaining of microscopic preparations which are then decolorized; it is necessary, therefore, to wash away completely all decolorizing fluid to prevent such fading.

To demonstrate nerve cells and axis-cylinders, silver impregnation methods also are best, and these usually are some modification of Bielschowsky's method. At the clinic a special

modification has been used for the last seven years and has been found to give satisfactory and uniform results. This method reveals changes in the neurofibrils of ganglion cells and degeneration of axis-cylinders, especially changes of the axis-cylinders of peripheral nerve fibers in so-called neuritis and similar conditions. Its chief advantages are that it can be done after formol fixation and paraffin embedding, it is comparatively simple, and, after a little experience, it is reliable and uniform (Method 4).

Some of the most valuable stains available to the neuropathologist are the Mallory stains and some of their modifications, especially the Mallory phosphotungstic acid-hematoxylin and the Mallory-Heidenhain stains for the study of neuroglia, connective tissue, and so forth. The Mallory phosphotungstic acid-hematoxylin stain makes connective tissue red to reddish-purple and glial tissue blue. This stain is particularly valuable in demonstrating neuroglial fibrils which stain blue (Method 5). The Mallory-Heidenhain stain is one of the most valuable stains in the study of peripheral nerves, spinal cord, pituitary gland, and so forth. This stain makes connective tissue sky-blue, myelin sheaths reddish-orange, and axis-cylinders purple. This stain is also very valuable in the study of tumors of the pituitary gland, since it brings out clearly the three types of cells. Mallory's ethyl-violet orange G stain can be used for the same purpose, but is not quite so satisfactory, and the colors not so vivid or distinctive. Tissue can be stained satisfactorily with these stains only when it has been fixed immediately after removal in Zenker's fixing solution for twenty-four hours. After this fixation sections must be treated with Lugol's solution to remove the bichloride of mercury which otherwise would be scattered as crystals diffusely over them.

These extremely valuable stains could not be used after tissue was fixed in formol solution, and refixing in Zenker's solution was not satisfactory; as a consequence, many attempts have been made to adapt formol-fixed material to Mallory's stains. A method has been used for some years at the clinic which has proved highly satisfactory for the adaptation of formol-fixed

material to Mallory's stains (Method 6). This method has continued to give good results since a report of it was published in 1931, and it will be found particularly useful for the demonstration of fibrin, neuroglia, fibroglia, and myoglia fibrils. It also brings out with great sharpness and faithfulness of detail the structure in mitotic figures, including those of spindles and centrosomes. Formol-fixed tissue may be deformolized by placing it in ammonia water for one to several days depending on the time the tissue had been in formol solution. It is then refixed in Zenker's solution for twenty-four hours or, better still, in Weigert's mordant I (Method 7) for forty-eight hours, and then transferred to Weigert's mordant II (Method 8) for four days; it is then embedded in paraffin. This tissue is then stained after the manner of Mallory or some of the modifications of his method.

An advantage is using Weigert's mordants after tissue has been fixed in formol solution is that the Weigert myelin-sheath stain can also be used. There are numerous modifications of this stain, but at the clinic the original Weigert stain has continued to be used because it has been found more uniform and constant than any of the modifications. This stain renders myelin bluish-black and all other tissue tan, except erythrocytes which are black (Method 9).

One of the traditional stains with which to demonstrate active degeneration in nerve tissue is the Marchi stain, but it has not been used at the clinic for some years because of the precipitation of crude osmium on tissue, which produced artefacts and led to misinterpretation, and also because of the difficulty of obtaining uniform penetration of this stain into the tissues. A preferable method of demonstrating such degeneration has been found to be the use of some of the fat stains, preferably Scharlach R, on frozen sections of formol-fixed material.

In recent years there has been a great increase in knowledge of the various types of glial cells, and this has been encouraged by better staining methods, particularly those of gold and silver impregnation. The larger glial cells, the astrocytes, which together with the blood vessels form the frame-work of the nervous system, are best demonstrated by Cajal's gold chloride

and sublimate method. This method is uniform, comparatively simple, and the results are excellent. It reveals cell bodies with all their ramifications and vascular attachments as well as intracellular fibrils. The original Cajal's method has not been improved on (Method 10). The results are unsatisfactory if the tissue has been fixed in commercial formol solution, so that a special fixative (the Cajal fixative) is necessary. The tissue is placed in this Cajal fixative for from four days to two weeks, and sections are cut frozen. The gold chloride solution must be made fresh each time it is to be used. If the solutions are properly made, and the tissue fixed in formol-bromine mixture, the results are uniform and excellent. The other two types of glial cells are not impregnated by the gold chloride method, but require impregnation by silver carbonate solution. These cells are (1) the oligodendroglia cells which are found in the cortex of the brain as satellite cells, and so forth, and are very numerous in white matter and (2) the microglia which represent the reticulo-endothelial cells of the brain and are the phagocytic cells of the central nervous system. These latter cells have numerous names: rod cells, gitter cells, compound granular corpuscles, scavenger cells, and so forth. Both these types of glial cells can be impregnated by the del Rio Hortega silver carbonate method after having been fixed in formol-bromine solution and after frozen sections have been made from the same block from which sections were cut to be stained with Cajal's gold chloride and sublimate method. There are several modifications of the Hortega method available, and these are slightly simpler and more uniform than the original method. At times, beautiful results can be obtained, but the chief objection to this method is the lack of uniformity in results; further work needs to be done both to simplify the procedure and to obtain more constant impregnation of cells. However, if care is taken in preparing the silver carbonate solution, so that all the precipitate is just dissolved and yet the solution is not too alkaline, the results will be satisfactory (Method 11).

There are numerous other staining methods that can be used in addition to these or to replace some of them, but only those

stains have been described which have been found at the clinic to be most suitable and reliable. The foregoing staining methods, however, are not always used for the study of all tissues, and only those stains are used which will be of help in demonstrating special cells or changes in definite disease processes; occasionally, therefore, additional methods are necessary. Several examples of stains used to demonstrate definite lesions are given so that they may be of help in indicating the uses of these stains.

In acute inflammatory lesions, such general stains as are used to demonstrate inflammation and its reactions in other parts of the body are usually sufficient. In chronic inflammation of the central nervous system, such as parenchymatous syphilis (general paresis), hematoxylin and eosin stain reveals the inflammatory and mesodermal reactions, but to demonstrate the changes in nerve cells and glial reactions, special methods are necessary. The thionin or toluidine blue stain demonstrates the degeneration of ganglion cells, also the fact that the normal architecture of the cortex is upset and that nerve cells are no longer in orderly arrangement; the modified Bielschowsky silver method will also demonstrate the disarrangement of ganglion cells. In order to appreciate the increased numbers of glial cells (astrocytes) Cajal's gold chloride and sublimate method is necessary. This method reveals marked gliosis in the cortex of the frontal lobes, whereas Hortega's silver carbonate method is necessary to demonstrate the increase in microglia, which are forerunners of most of the scavenger cells of the cerebrum. Any iron stain will reveal the well-known fact that, in paresis, there is a considerable deposit of iron in the cortex. If it is desired, Pappenheim's stain for plasma cells will indicate that many of the inflammatory cells in the perivascular spaces are plasma cells.

In recent years, there has been a marked increase in our understanding of that group of tumors under the heading "gliomas," and neurologists and neurosurgeons request more specific information as to type because on this further classification depends the extent of the operative procedure and of the radium or roentgen-ray treatment, and the ultimate prognosis. In the identification of various gliomas, special staining methods are valuable

and are necessary to substantiate the opinion arrived at by using the general stains. At the clinic, hematoxylin and eosin is the routine stain for all tumors. When possible, small portions of the tumor are fixed in Zenker's solution and in formol-bromine mixture. Cajal's gold chloride and sublimate method is useful since it demonstrates the astrocytes of astrocytomas, the astroblasts of astroblastomas, and also the several types of spongioblasts; this method reveals the dominant-cell type in some of the mixed types of gliomas. Mallory's phosphotungstic acid-hematoxylin stain demonstrates neuroglial fibrils in astrocytomas and distinguishes them from other types of gliomas; it is also helpful in demonstrating blood vessels and such changes as the proliferation of lining endothelium of the dilated, thin-walled blood vessels of rapidly growing gliomas. This stain is also valuable in distinguishing connective tissue from neoplastic tissue in meningiomas. The Perdrau silver impregnation method for connective tissue gives a beautiful outline of the blood vessels and blood spaces in hemangio-endotheliomas and, when done on frozen sections in conjunction with a fat stain, it shows the relation of fat-containing endothelial cells to the blood spaces of these tumors. Hortega's silver carbonate method for oligodendroglia is useful, at times, in demonstrating these cells in oligodendrogliomas, but it is unsatisfactory as a general rule and not very reliable.

In some chronic degenerative diseases of the central nervous system, such as multiple sclerosis, combined degeneration (usually associated with pernicious anemia), tabes dorsalis, amyotrophic lateral sclerosis, and so forth, special staining methods are necessary to reveal the specific changes that are diagnostic. In tabes dorsalis, the degenerative condition of the posterior columns can only be shown well with a myelin sheath stain. When this stain is used, the anterior and most of the lateral columns will be blue-black, whereas posterior columns, peripheries of the lateral columns, and gray matter, will be tan. Mallory's phosphotungstic acid stain, which gives excellent results after the Weigert's mordants have been used, and can thus be used on sections cut from the same blocks and at the same

time that the sections for the Weigert stain were cut, stains blue the posterior columns and peripheries of the lateral columns, indicating an excess of glial cells and of neuroglial fibrils. Scar tissue of the central nervous system is neuroglia and, in tabes dorsalis, multiple sclerosis, and so forth, represents replacement of degenerated myelin. The modified Bielschowsky impregnation method on longitudinal sections of the spinal cord reveals that, in tabes dorsalis, axis-cylinders have disappeared from the areas in which myelin has been destroyed, whereas in multiple sclerosis, they are preserved except in the oldest and most advanced lesions. This method gives excellent results when done on frozen sections in conjunction with a fat stain in cases of recent injury or active degeneration where the myelin has degenerated into neutral fat and axis-cylinders are actually degenerating. Many other methods may be used for diseases of the spinal cord, but the foregoing usually are sufficient except in cases of meningitis, myelitis, or poliomyelitis, where general stains show the inflammatory reaction and toluidine blue or thionin stains reveal the degeneration of large anterior horn cells. Cajal's and Hortega's methods are not so valuable in studies of the spinal cord as in those of the brain. The same staining methods, however, apply to tumors of the spinal cord as to those of the brain. There are many diseases involving the peripheral nerves, but by far the most common of these is so-called peripheral neuritis with its numerous clinical subdivisions. True inflammation of peripheral nerves is extremely rare, whereas degenerative conditions are much more common. The changes in degenerative lesions of peripheral nerves can readily be demonstrated. The modified Bielschowsky silver impregnation method reveals the swelling, beading, and fragmentation of the axis-cylinders, whereas although Weigert's myelin-sheath stain shows changes of the myelin sheaths the Mallory-Heidenhain stain has been found at the clinic to be a better one. This stain can be applied best after the use of Weigert's mordants, or after Zenker's solution and Weigert's mordant II, and it shows myelin reddish-orange, connective tissue blue, and axis-cylinders pale-purple. By using this stain, the blood vessels are seen easily and it is on

the basis of vascular changes that most of the degenerative conditions of the peripheral nerves depend. The Mallory-Heidenhain stain is better for studying lesions of peripheral nerves than those of the central nervous system. The hematoxylin and eosin stain will also show inflammatory changes if any are present, and van Gieson's stain will reveal any increase of connective tissue or increase of the thickness of the walls of blood vessels with attendant narrowing of the lumens. On this basis most so-called neuritis occurs, and it should more properly be called ischemic neuritis.

SUMMARY

Since many text books have been devoted to neurologic technic, it is obvious that the above methods are very incomplete, but by their use, most lesions of the nervous system can be detected and a correct diagnosis reached. The methods presented in this paper are not the only ones, or necessarily the best ones, but I have found them useful, for several years. Most of them could be replaced by others equally good and satisfactory. It is not the object of this small contribution to replace the text books; the purpose has been rather to simplify the methods and to make possible the study of neurologic tissue in any laboratory without the necessity of elaborate equipment or of very complicated staining methods. With a little experience these methods will be found to be quite reliable, and reactions in the nervous system to inflammation and disease will be fairly easy to demonstrate and to understand.

APPENDIX

Method 1

Fat staining method

1. Fix tissue in formol solution, freeze and section, 10 to 20 microns.
2. Wash sections in 70 per cent alcohol for one minute.
3. Stain for five minutes in fat stain, made as follows:
   Sharlach R, 1 gm. (sudan IV may also be used)
   Absolute alcohol, 70 cc.
   Distilled water, 30 cc.

(Heat this mixture just a little, and shake while heating. Then add 100 cc. acetone and put in a 37°C. oven overnight. Always filter stain before using.)

4. Wash in 70 per cent alcohol quickly.
5. Stain in hematoxylin (Harris) for five minutes.
6. Wash in tap water.
7. Dip in (1 per cent) acid (70 per cent) alcohol about two seconds.
8. Wash in tap water ten minutes or longer.
9. Wash in lithium carbonate (saturated aqueous solution) about two seconds.
10. Wash in tap water and mount with glycerine.

Method 2

Perdrau impregnation method

1. Paraffin sections run down to water in usual way.
2. Place for twenty to thirty minutes in 0.25 per cent solution of potassium permanganate.
3. Wash in water.
4. Place in solution of equal parts of 1 per cent oxalic acid and 1 per cent potassium sulphite, or 5 per cent oxalic acid, until color is gone.
5. Wash for a half to one hour in tap water until all acid is washed out (limit the length of time in case the sections come loose).
6. Place sections in a 20 per cent solution of silver nitrate for half an hour.
7. Wash quickly in double-distilled water.
8. Place sections in the following solution:
   Silver nitrate (20 per cent) solution, 5 cc.
   Sodium hydroxide, 6 drops.
   (Add ammonium hydrate drop by drop until the precipitate is almost destroyed. Add distilled water up to 50 cc. and filter. Leave sections in this solution for half an hour.)
9. Wash in double-distilled water.
10. Fix in 10 per cent formol solution (neutral).
11. Wash in double distilled water.
12. Tone in gold chloride solution 1–500.
13. Fix in sodium hyposulphite.
14. Wash in tap water, dehydrate, clear, and mount.

Method 3

Toluidine blue, or thionin, staining method

1. Run sections down to water.
2. Place sections in toluidine blue or thionin, stain for fifteen to twenty minutes (heat stain before using for thirty minutes):
   1:500 solution = 1 gm. toluidine blue or thionin to 500 cc. water.
3. Wash in two changes of 95 per cent alcohol (color comes out rapidly).

4. Place in absolute alcohol and watch under microscope until differentiation is complete.
5. Clear in xylol, thoroughly, and mount with Canada balsam.

Method 4

Modified Bielschowsky impregnation method

1. Fix tissue in solution of formol, 10 per cent.
2. Embed section in paraffin and cut at 10 microns.
3. Deparaffinize.
4. Wash three times in double-distilled water.
5. Place in 20 per cent solution of silver nitrate for one hour.
6. Wash quickly (twice) in double-distilled water.
7. Transfer sections into ammoniated solution of silver nitrate for five minutes:
   To 40 cc. of 20 per cent silver nitrate solution add ammonium hydrate drop by drop until precipitate is dissolved. An excess of ammonium hydrate is noxious. Make a fresh solution each time it is to be used. Filter before using.
8. Wash quickly in double-distilled water.
9. Transfer sections into 10 per cent solution of formol (neutral) for one minute.
10. Wash, tone in gold chloride (brown) solution (a), fix in sodium hyposulphite solution (b), one or two minutes, clear (c), and mount (d):
    (a) Gold chloride, 1 gm.
    Water, 500 cc. (can be kept as a stock solution)
    (b) Sodium hyposulphite, 5 gm.
    Water, 100 cc.
    (c) 95 per cent alcohol
    Acetone
    Carbo-xylol
    Xylol
    (d) With Canada balsam

Method 5

Mallory-Heidenhain's staining method

1. As a fixative, use formol or Zenker's solution, or Weigert's mordants. Cut paraffin sections at 5 microns.
2. Stain forty minutes in:
   Azo-carmine (Grubler's), 1 gm.
   Water, 100 cc.
   (Heat, cool, and filter at room temperature and add 1 cc. of glacial acetic acid before using.)
3. Wash in water.

4. Differentiate in aniline alcohol (watch under microscope) until nuclei are red and cytoplasm is pale-pink. This step requires from three to ten minutes depending on thickness of sections.
5. Remove aniline alcohol with acid alcohol (1 per cent) a half to one minute.
6. Wash quickly in water.
7. Place in 5 per cent phosphotungstic acid for three hours.
8. Wash quickly in water.
9. Stain one-fourth to one-half hour in Mallory's aniline blue made as follows:
   Aniline blue, 0.5 gm.
   Orange G, 2 gm.
   Water, 100 cc.
   Acetic acid, 8 cc.
   (Boil, cool, filter, and thin with equal parts of water before using.)
10. Wash quickly in water.
11. Differentiate in absolute alcohol.
12. Clear in carbo-xylol and xylol.
13. Mount with Canada balsam.

Method 6

Mallory's phosphotungstic acid-hematoxylin staining method

1. Fix in Zenker's solution for twenty-four hours.
2. Place in running water for twenty-four hours.
3. Embed in paraffin.
4. Section and deparaffinize.
5. Place in potassium permanganate for from five to twenty minutes (make a fresh 0.25 per cent aqueous solution each time).
6. Wash well in water.
7. Place in oxalic acid (5 per cent aqueous solution) for from five to ten minutes.
8. Wash thoroughly in several changes of water.
9. Stain with Mallory's phosphotungstic acid-hematoxylin stain three to twelve hours. This stain is made as follows:
   Ammonia hematin, 0.1 gm.
   Water, 100 cc.
   Phosphotungstic acid crystals (Merck), 2 gm.
   (Dissolve the ammonia hematin in a little water, with the aid of heat, and add it when cool to the rest of the solution; no preservative is required. If the solution stains weakly at first, it may be ripened by the addition of 5 cc. of a 0.25 per cent aqueous solution of potassium permanganate, or it may be allowed to stand for a few weeks until it ripens spontaneously. Hematoxylin may be used instead of ammonia hematin, but requires 10 cc. of the permanganate solution to ripen it).
10. Place sections in 95 per cent alcohol, then in absolute alcohol, and clear in xylol.
11. Mount with Canada balsam.

METHOD 7

Weigert's mordant I

Potassium bichromate, 5 gm.
Fluorochrome, 2 gm.
Water, 100 cc.
(Fix tissue in mordant I for from four to five days, for small and thin sections, two days.)

METHOD 8

Weigert's mordant II

Acetate of copper, 5 gm.
Fluorochrome, 2.5 gm.
Acetic acid (36 per cent), 5 cc.
Water, 100 cc.
Formol, 10 cc.
(Fix tissue in mordant II for from twenty-four to forty-eight hours.)

METHOD 9

Weigert's myelin-sheath staining method

1. Wash tissue, which has been fixed in 10 per cent solution of formol, in water for short time.
2. Fix in primary mordant four to five days (for small and thin sections, two days).
3. Wash in water one hour.
4. Fix in secondary mordant twenty-four to forty-eight hours.
5. Wash well in water one to two hours.
6. Embed sections and deparaffinize.
7. Stain two hours in a solution made as follows:
   10 cc. of a 10 per cent solution of hematoxylin dissolved in absolute alcohol
   Saturated solution of lithium carbonate, few drops
   Distilled water, 90 cc.
   (Make this solution fresh each time)
8. Wash in tap water.
9. To differentiate, place in a solution of:
   Borax, 2 gm.
   Potassium ferricyanide, 2.5 gm.
   Water, 100 cc.
10. Wash thoroughly in running water.
11. Dehydrate in 80 per cent and in 96 per cent alcohol.
12. Clear in acetone, carbo-xylol, and xylol.

13. Mount with Canada balsam.
    (A stock solution of 10 per cent solution of hematoxylin in absolute alcohol should be kept on hand. At least ten days' exposure to sunlight is required to ripen this solution. Combine it with a few drops of saturated aqueous solution of lithium carbonate at the time of using.)

Method 10

Cajal's gold chloride and sublimate method

1. Place small portions of fresh tissue in the following solution for two to ten days (the tissue may have previously been fixed in formol solution a very short time):
   Formol (neutral), 15 cc.
   Ammonium bromide, 1.5 to 2 gm.
   Distilled water, 85 cc.
   (Tissue should not remain more than ten days in this solution, seven days is best)
2. Cut sections on freezing microtome (20 to 25 microns) and place in 6 per cent neutral formol solution.
3. Wash a few moments in distilled water.
4. Place in following solution for six to ten hours and keep in the dark:
   Gold chloride (1 per cent), 10 cc.
   Mercuric chloride, 0.5 gm. (10 cc. of a 5 per cent solution)
   Distilled water, 60 cc.
   (Dissolve the mercuric chloride by heat in nearly all water called for in the formula, filter, and add to the gold chloride. The best temperature is 18° to 20°C. (65° to 68°F.) At this temperature, four to six hours is the best.
   If temperature is 14° to 17°C., the time should be much longer. If temperature is higher, the time should only be two to three hours.)
5. After four or more hours, when sections are intense purple, fix for from six to ten minutes in the following solution:
   Sodium hyposulphite, 5 cc.
   Alcohol (95 per cent), 30 cc.
   Distilled water, 70 cc.
   Saturated solution bisulphite of soda, 5 cc.
6. Wash in 50 per cent alcohol.
7. Place on slide, blot carefully, then dehydrate on slide with 70 per cent, and 95 per cent, and absolute alcohol; then clear in xylol.
8. Mount with Canada balsam.
   (Blot carefully after each of above steps. Oil of origanium may be used in place of absolute alcohol.)

METHOD 11

Hortega silver carbonate impregnation method for oligodendroglia and microglia

1. Fix tissue in formol-ammonium bromide solution (same as for Cajal's stain) and cut frozen sections.
2. Wash in two dishes of distilled water, the first containing 10 drops of ammonia.
3. Stain in undiluted silver carbonate for from one minute to one and a half hours. Del Rio Hortega's undiluted ammonical silver carbonate is prepared as follows:
   Solution of silver nitrate (Merck), 10 per cent, 5 cc.
   Solution of sodium carbonate (pure), 5 per cent, 20 cc.
   Ammonium hydroxide, sufficient to dissolve precipitate
   (The ammonium hydroxide should be added drop by drop until the precipitate is just dissolved, stirring the solution all the while.) Finally, filter the solution and place in a dark bottle, where it will keep for long periods.
4. Wash rapidly in 60 per cent alcohol (made by using absolute alcohol). The section should be carried through with a small angulated glass rod so as to allow all of it to be washed equally without wasting time. If the section is wrinkled or folded, the alcohol will produce a patchy result.
5. Reduce by passing sections directly in 1 per cent formol solution.
6. Wash in distilled water.
7. Tone by placing sections in a gold chloride bath ten to fifteen minutes until they become purple-gray.
   Gold chloride (yellow), 1 gm.
   Distilled water, 500 cc.
8. Fix in a 5 per cent solution of sodium hyposulphite for half a minute or more until sections are flexible.
9. Wash in water.
10. Dehydrate in dishes of graded alcohol and clear in carbo-xylol and xylol.
11. Mount with Canada balsam.

FURTHER STUDIES IN ANTIGEN EMULSION PREPARATION FOR THE BALL TEST FOR SYPHILIS*

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Several years ago a ball test for syphilis was described.[2] The results with this test are very easy to read accurately with the naked eye. The negative test shows a uniformly turbid fluid. A strongly positive test on the other hand shows a thoroughly clear fluid and suspended in its upper portion a large opaque white ball. A positive test shows a few large white clumps in the upper half of a slightly turbid fluid and a doubtful test shows numerous small white clumps suspended in turbid fluid. The phenomenon of ball formation in this test is apparently dependent upon the interlocking of numerous small clumps of needle like particles which dovetail into each other upon centrifugation. The ball test applied in two slightly different ways gives results of about equal sensitivity with the two microscopic slide precipitation tests (1, for the diagnosis and 2, for the exclusion of syphilis) and serves as an excellent check for the slide tests.

The antigen emulsion for the ball test as described is prepared by mixing salt solution and cholesterinized antigen, the resultant combined antigen cholesterin particles although of desirable shape (long thin needles) are satisfactory for use for only a few to several hours after preparation following which time the emulsion breaks down, the particles become granular and the globules of antigen only present in the emulsion clump together.

As described previously,[2] emulsions generally employed in precipitation tests for syphilis (including the ball test mentioned above) are made by mixing salt solution with cholesterinized antigen. The resultant aggregates are composed of antigen

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lipid, cholesterin and adventitious substances more or less uniformly distributed throughout the particles. In addition there are a number of globular particles of antigen only. The size and shape of the combined antigen cholesterin particles and particles of antigen only are determined especially by the quantitative relationship of lipid and water, by the temperature of the ingredients and by the speed at which the mixture is made. In emulsions chemically identical for instance the particles vary from less than 1μ in diameter when very warm ingredients are quickly mixed to large needle like particles 60μ or more in length when very cold ingredients are slowly mixed. The particles furthermore undergo continuous change in size and shape and in a few minutes to several hours are unsatisfactory for use in tests for syphilis.

In contrast to such emulsions, those employed in the microscopic slide precipitation tests for syphilis are made by first precipitating the cholesterin crystals in water and subsequently coating them with antigen lipid. Cholesterin plates precipitated from alcoholic solution by a small or large amount of warm or cold water or salt solution, by slow or by rapid mixture vary but little in size and shape. Upon this fact depends the greater uniformity in particle type and of sensitivity of slide test emulsions in routine practice. Furthermore the particles maintain their size and shape for over a week and the emulsions containing them are thoroughly satisfactory for use for at least 48 hours after preparation.

It is apparent therefore that the ball test for syphilis previously reported can be improved by preparing stable needle like particles to hold antigen on their surfaces. This has been found possible by two methods:

(1) Addition of about equal parts of Squibb's anesthetic ether to 1 per cent alcoholic solution of cholesterin and the addition of cold water to this combined solution after it has been chilled.

(2) Addition of 4 parts chilled 0.5 per cent cholesterin solution in glacial acetic acid to 1.75 parts chilled 60 per cent alcohol.

The needles prepared by the ether method are rather fragile and rather sensitive to an excess of electrolytes. The needles

prepared by the acetic acid method are most satisfactory in this regard and furthermore after they are removed from the acetic acid and alcohol solution and properly neutralized with 7 per cent sodium hydroxide they serve excellently in holding antigen on their surface. Emulsions containing these antigen coated needles in proper proportions with serum (about 1 part emulsion, 5 parts serum) have given results similar to the ball test previously described and have been found satisfactory for use for at least 24 hours after preparation. It is hoped before long to have all the details of emulsion preparation and test performance worked out.

In addition to these studies with antigen coated cholesterin needles some experiments have been made using globules of antigen only as in the Hecht[1] ball test. In that test antigen globules are precipitated from alcoholic solution by about a half part of salt solution and after a ten minute period of ripening another half part of salt solution is added. At this time as after the first step the antigen globules are in small clumps which are dispersed by the patient's serum whether positive or negative. In addition to the globules, which vary greatly in size from less than 1μ to about 25μ there are many elongated tongue shaped particles and these readily dovetail into each other in positive tests.

Since the size and shape of particles of antigen only in water or salt solution are influenced by so many factors the possibility of preparing uniformly sensitive ball test emulsions with antigen alone is not as great as with antigen coated needles of uniform size and shape.

SUMMARY

In the ball test for syphilis described several years ago the emulsion particles of combined antigen and cholesterin are unstable and satisfactory for use for a few to several hours only.

Further studies have demonstrated the possibility of preparing emulsions with stable antigen coated cholesterin needle like crystals that are satisfactory for use in the ball test for at least 24 hours.

REFERENCES

(1) Hecht, Hugo: Colored flocculation reactions. Proc. Soc. Exper. Biol. and Med., **31:** 849–852. 1934.

(2) Kline, B. S.: Mechanism of the microscopic slide precipitation tests for syphilis. Jour. Lab. and Clin. Med., **16:** 1202–1216. 1931.

CYANIDE POISONING*

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Hydrocyanic acid as a poison was said to have been used by the ancients, usually in the form of an infusion of peach stones or seeds of other fruits in which hydrocyanic acid was liberated. However, accurate records on this subject are not available. One of the earliest authentically recorded cases occurred in Germany in 1781.[4,5] For a time in the early and middle parts of the last century the cyanides were said to have been a popular method for committing murder and suicide in this country, but here again accurate statistics were not kept.

In recent years suicidal, accidental and industrial cyanide poisonings have occurred with increasing frequency. Homicidal attempts have been rare. Deliberate poisonings have usually been produced by mixing potassium or sodium cyanide in food, drink or medicines. Accidental poisonings result chiefly from fumigation, mistaking a container of cyanide for a medicine, in chemical laboratories and in certain industrial occupations. The industrial type results from the escaping vapors in certain chemical processes, notably the silver, gold, celluloid and polishing industry. Domestic animals (goats, sheep, cows) and even man are found now and then either dead or suffering from symptoms of cyanide poisoning as a result of the ingestion of fruits sprayed with an insecticide containing cyanide or from plants that are relatively rich in the glucosid amygdalin (peach leaves, cherry, laurel, et cetera). The latter is broken down into sugar, oil of bitter almonds and hydrocyanic acid in the presence of water

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and emulsin. It is of considerable practical significance that hydrocyanic acid is a poison for all members of the animal kingdom[8] (rodents, flys, bugs, et cetera). Its use as a lethal gas in the late war was not as successful as were some of the other gases. Its use as a method for legal execution has not gained much popularity.

McKelway[7] records an interesting case in which a hairdresser, aged 38 years, attempted to remove "silver discoloring" of hair dye from her hands by rubbing them with potassium cyanide. At first the patient developed symptoms of vertigo, nausea, et cetera (probably caused by inhalation of cyanide) which disappeared only to recur about one and one-half hours later in a much more aggravated form from which the patient, however, recovered. Absorption of cyanide through the skin was thought to have taken place, though it was not noted whether she had washed her hands free of the salt after her first attack. Experimental absorption of hydrogen cyanide through the skin has been proved and Drinker[1] has observed it in human beings. Accidental poisonings through the injured skin have been frequent and recently mention has been made of poisoning developing from silverware used at table which had been cleaned with a polish containing cyanide, the utensils not having been properly washed.

SYMPTOMS

The symptoms of acute poisoning proceed with almost lightning-like rapidity. Within two to five minutes after ingestion of the poison, the individual collapses, frequently with a loud scream (death scream); there are a few short dyspneic inspirations followed by prolonged expirations; trismus and perhaps tetanus may be observed; bloody, frothy saliva and vomiting may occur. In cases, especially as a result of admixture with foods, where absorption is not quite as rapid, Lewin[6] divides the symptoms of cyanide poisoning into three stages, as follows:

(1) *Dyspneic stage.* Sense of constriction in the throat, oppression, fear, staggering walk, nausea or vomiting, headache, vertigo, weak pulse, prolonged respirations with short inspirations and prolonged expirations, and the intervals between breaths becoming longer and longer.

(2) *Stage of convulsions.* The patient falls, the pupils are dilated, and the skin is covered with a cold clammy perspiration. The pulse becomes more rapid, convulsions, opisthotonus, trismus and involuntary micturition ensue.

(3) *Stage of asphyxia.* Respirations become irregular and prolonged, the heart irregular and slow, the body temperature drops, cyanosis of face and extremities is noted, the patient sinks into a coma and frothy, often blood-tinged saliva flows from the mouth. Death usually follows within a half hour.

In some patients who have recovered from poisoning, complaints of weakness, fatigue, insomnia and cardiac distress continuing for months are occasionally encountered.

The symptoms of chronic cyanide poisoning vary considerably with each person, dependent apparently on individual idiosyncrasies and susceptibilities. Some individuals by merely entering a place in which cyanide is handled, quickly develop symptoms of nausea, headache, giddiness and scratching or irritability of the throat. Others may work in an atmosphere containing cyanide in comparative comfort or develop symptoms only after prolonged exposure or contact. In a well developed case of chronic poisoning, the patient usually complains of lassitude, easy fatigue, attacks of nausea and vertigo, occasional vomiting, headache, insomnia, cardiac discomfort, anemia, skin eruptions, and occasionally ocular and auditory disturbances. The vagueness of the symptoms makes a diagnosis at times exceedingly difficult, unless the patient's occupation yields a clue. In the combustion of tobacco during smoking appreciable amounts of hydrogen cyanide vapors are formed, though nothing at present is definitely known as to its effect in intensive smokers.

The action of cyanide in the body is described as interfering with the "internal" cell respiration by paralysis of the oxydase ferments. Thus oxidation and reduction cannot continue in the cells. Hanzlik and Richardson[3] experimentally and Geiger[2] clinically have used a 1 per cent solution of methylene blue (containing 1.8 per cent sodium sulphate) in cases of cyanide poisoning with good results. The basis of the action of methylene blue is to convert the hemoglobin into methemoglobin which then

combines with the cyanide forming cyamethemoglobin, which is innocuous.

NECROPSY

Postmortem examination of an acutely poisoned subject frequently shows bright red spots in the skin and cyanosis of the face, neck and extremities. On opening the body the odor of hydrogen cyanide is distinct and almost invariably noticeable. The blood, depending on the number of hours postmortem, is generally quite dark, remains fluid and engorges the veins and right heart. Frequently the mucous membrane of the lips, mouth and esophagus, especially if the poison is taken without an admixture of food or drink, is swollen and shows deep reddish discoloration; rarely corrosion may occur. The stomach shows diffuse edematous swelling of the mucous membrane, with reddish punctate discoloration of the surface, often accentuated corrugation of the surface and considerable exuded mucus due to the hydrolysis of the alkali cyanide salts. Corrosive ulceration is rare. A similar but generally very much less marked process is found in the duodenum and occasionally even in the trachea. In the serous cavities, especially in the pericardium, punctate ecchymoses may be found and in the pia arachnoid and ventricles an edematous bloody extravasation occurs. The liver, especially if the patient has survived any length of time, may show fatty degeneration and the urine at times shows some blood.

In cases where death follows inhalation of cyanide gas, the lesion is not marked in the alimentary tract; instead there are swelling and edema of the mucous membrane of the respiratory tree with ecchymoses or hemorrhages and pulmonary edema and congestion. Lewin mentions a case of cyanide gas poisoning where degeneration of the ganglion cells, lesions of the blood vessels, thromboses and hemorrhage were found.

Histologically, in the acute poisonings, edema of the mucosa of the stomach or bronchi with circumscribed hemorrhages and leucocytic infiltration are found. Edema and miliary hemorrhages and occasionally perivascular extravasations of blood are found in the pia, brain substance and in the cord. In the chronic

cases, fatty degeneration and round cell infiltration in the small vessels, especially of the brain, are noted. Occasionally calcium deposits, hyaline degeneration and thrombus formation in the vessels are noted. In chronically poisoned animals, chromatolysis, vacuolization and degeneration of the central and peripheral nervous system have been found.

In the office of the Chief Medical Examiner of the City of New York, from January 1, 1918 to December 31, 1933, there were

TABLE 1

YEAR	HOMICIDES	SUICIDES	ACCIDENTAL (INGESTION OF CYANIDE)	ACCIDENTAL (FUMIGATION WITH CYANIDE)	TOTAL NUMBER
1918		8		2	10
1919		3		13	16
1920		10		8	18
1921		28		7	35
1922		10		4	14
1923		18		5	23
1924		18	4		22
1925		18		4	22
1926		13	1	3	17
1927		12		1	13
1928	1	18	7		26
1929	2	22	2	1	27
1930		34	2	1	37
1931		24	1		25
1932	1	44	4		49
1933		49	4	3	56
Totals.....	4	329	25	52	410

410 cases of death from cyanide poisoning. In so far as an analysis of the records permit, they are distributed as in table 1.

The deaths due to fumigation resulted from fumigation of ships, hotels and warehouses. Of the other accidental deaths, the majority were caused by mistaking the poison for medicine.

TOXICOLOGICAL ANALYSIS

Organs best suited for analysis

If the poison has been taken by mouth, the stomach contents and brain should be analyzed. Analysis of the brain is necessary for the purpose of ruling

out the possibility of the poison having been introduced into the stomach after death. If the poisoning resulted from inhalation, the lungs and brain must be examined. In cases of poisoning by inhalation, usually none, or only the very faintest trace, is found in the stomach contents. This is of tremendous importance from the medicolegal aspect.

Method of isolation of cyanide

The tissues are cooled by keeping them in an ice-box. Two hundred to 500 grams of tissue are ground up. Care should be taken to keep the tissue cold since hydrocyanic acid may volatilize if warm. If stomach contents are analyzed, usually one-fifth of the total volume of the contents is used. The ground-up tissue or the stomach contents are placed in a one liter flask and acidified with tartaric acid. The material is then distilled with steam, using a well cooled condenser the tip of which is bent to serve as an adapter and dipped into 5 cc. of 5 per cent sodium hydroxide solution in a receiving flask. The latter should be packed in ice. One hundred cubic centimeters of distillate are collected, which is ample to recover all the cyanide present, the following tests being employed.

Qualitative tests

(1) *Schönbein's test.* Suspended a strip of filter paper, impregnated with guaiac and copper sulphate, over the material in a flask, the paper being held in place by the stopper. (Dip strip of filter paper into a freshly prepared alcoholic solution of guaiac 1:10; then let dry; when dry, moisten it with dilute (1:10,000) copper sulphate solution). If color does not change, cyanide is absent and no further tests need be made. If a blue color results, cyanide *may* be present. The test is very sensitive but not specific, hydrochloric acid, nitric acid, chlorine, bromine, ozone, hydrogen peroxide, as well as some other substances also give a positive test. This test may be used as a preliminary one at the necropsy table. The following two tests must be employed since they are specific for cyanide:

(2) *Prussian blue test.* To 5 cc. of distillate, add 3 cc. of 25 per cent sodium hydroxide, then a few drops of freshly prepared ferrous sulphate solution and a few drops of ferric chloride solution. Warm a little. Let cool and add concentrated hydrochloric acid, drop-wise, until the dirty brown precipitate just dissolves; avoid excess hydrochloric acid. If cyanide is present, a deep blue precipitate (Prussian blue) appears. If only a trace of cyanide is present, a green solution results instead of a blue precipitate, but, on standing several hours, a small flocculent Prussian blue precipitate settles (sensitive to one part in 50,000).

(3) *Liebig's test.* To 10 cc. of distillate, add 1 cc. of yellow ammonium sulphide and evaporate to dryness on the water bath. When dry, add 5 cc. of 5 per cent hydrochloric acid solution, warm a little and stir well to dissolve all of the thiocyanate that was formed during the evaporation. Let stand two hours,

then filter. To the filtrate, add 5 to 10 drops of 10 per cent ferric chloride solution. If cyanide is present, a deep red color results (sensitive to one part in 10 million).

The following tests may be used, but they are not specific for cyanide:

(4) *Vortmann's test.* To 5 cc. of distillate, add a few drops of potassium nitrite solution, then 2 to 4 drops of ferric chloride solution and then enough dilute sulphuric acid until the color of the solution becomes a bright yellow. The solution should then be boiled, after which cool and add ammonium hydroxide until all of the iron is precipitated. Filter off the precipitate. To the filtrate add a few drops of a very dilute solution of ammonium sulphide. If cyanide is present, a play of colors results, violet, blue, green, yellow.

(5) *Picric acid test.* To 5 cc. of distillate (slightly alkaline) add a few drops of picric acid solution and warm gently. If cyanide is present, a red color develops (sensitive to one part in one million).

(6) *Phenolphthalin test.* To 5 cc. of distillate, add a few drops of alkaline phenolphthalin solution (reduced phenolphthalein), then a few drops of 1:2000 copper sulphate solution. If cyanide is present, a red color develops (sensitive to one part in 20 million).

(7) *Silver test.* To 2 c.c. of distillate, add nitric acid until reaction is acid, then add a few drops of silver nitrate solution. If cyanide is present, a white precipitate of silver cyanide results.

Quantitative test

For quantitative analysis, a weighed amount of tissue is distilled, as described in the qualitative procedure. In the receiving flask, however, instead of having dilute sodium hydroxide, 20 cc. of 10 per cent silver nitrate solution are used, acidified with nitric acid. In order to make certain that all of the cyanide is isolated, distillation is continued until 200 cc. of distillate are obtained. During the distillation, the cyanide precipitates as silver cyanide. This is then filtered through a previously weighed Gooch crucible, washed, dried and weighed. From the weight of the silver cyanide, the amount of cyanide in the material analyzed is calculated as HCN.

In quantitative analysis, if the poison was taken by mouth, the entire amount of cyanide is determined in the gastro-intestinal tract. This is then multiplied by 100/98, which gives the amount in the entire body. This fraction is used because approximately 98 per cent of the cyanide, if taken by ingestion, remains within the stomach contents. If the poison was introduced by inhalation or injection, parts of all the organs and tissues are analyzed and, from these results, the amount present in the entire body is calculated.

The lethal dose of cyanide is accepted as 50 mgm., calculated as hydrocyanic acid.

The following factors interfere with the determination of the presence of cyanide:

(1) Traces of hydrogen cyanide are produced during the first few days of putrefaction, but this disappears in the later stages.

(2) Cyanides present in the tissues disappear during prolonged putrefaction and are changed to sulphocyanide.

(3) In stomach contents, where the bulk of the cyanide remains at death, putrefaction is of little importance.

(4) Embalming with formaldehyde interferes greatly in the tests, the cyanide forming condensation products with the formaldehyde.

It is, therefore, important that early toxicological analysis be made in cases of suspected cyanide poisoning. It is likewise necessary to rule out the presence of ferrocyanides, ferricyanides and thiocyanates before distillation is begun, because these compounds, when distilled in the presence of mineral acids, yield hydrogen cyanide.

Diffusion of the poison in a body after death has been recorded, but the process is extremely slow and it has been shown by Gettler, that, when cyanide is introduced into the stomach after death, no cyanide reaches the brain even after an interval of two months.

Although the term "cyanide of potassium" is generally employed in referring to cyanide poisoning, it is not the potassium salt but, because of its cheaper cost, the sodium salt that is used.

SUMMARY

(1) Four hundred and ten cases of cyanide poisoning are listed.

(2) The relative incidence over a period of fifteen years is noted.

(3) The pathology and mechanism of the action of cyanide is described.

(4) The tests for its chemical identification and the importance of differentiating cases of poisoning by inhalation and by ingestion are described.

REFERENCES

(1) DRINKER, P.: Hydrocyanic acid gas poisoning by absorption through the skin. Jour. Indus. Hygiene, **14**: 1–2. 1932.

(2) GEIGER, J. C.: Cyanide poisoning in San Francisco. Jour. Am. Med. Assn., **99**: 1944–1945. 1932.

(3) HANZLIK, P. J., AND RICHARDSON, A. P.: Cyanide antidotes. Jour. Am. Med. Assn., **102:** 1740–1745. 1934.

(4) VON JOSCH, J. R.: Der Fall Thomas Maschek (vergifteter Messwein). Arch. f. Krim.-Anthrop. u. Kriminalist., **14:** 90–107. 1903–4.

(5) LEWIN, LOUIS: Die Gifte in der Weltgeschichte. Berlin: J. Springer, 1920, pp. 596.

(6) LEWIN, LOUIS: Gifte und Vergiftungen. p. 505. Berlin: Georg Stilke, 1929.

(7) MCKELWAY, J. I.: Three cases of poisoning by potassium cyanide. Am. Jour. Med. Sci., **129:** 684–688. 1905

(8) ROBERTS, NORMAN: Cyanide fumigation of ships; method used at New Orleans. Public Health Rep., Washington, 50, **29:** 3321–3325. 1914.

TREATMENT OF MALIGNANT NEUTROPENIA BY INJECTION OF LIVER EXTRACT*

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Since Schultz[9] and Friedemann[7] reported their cases of the peculiar disease characterized by ulcerating and necrosing lesions of the throat and a marked decrease in the leukocytes especially in the granulocytic cells, there have been an increasing number of cases of a similar character reported each year. This increasing incidence, together with the high mortality rate, lends interest to any suggestion that might lead to more effective treatment.

Experience in treating three cases with liver extract is the basis of this report. The usual liver preparation for oral and intramuscular treatment of pernicious anemia has been used. Intravenous injections have not been given. The usual procedure has been to give the equivalent of 300 gm. of liver by mouth and the equivalent of 100–200 gm. of liver intramuscularly each day. With the appearance of satisfactory improvement, the intramuscular injections have been discontinued and the treatment continued orally.

The first case treated was seen in March, 1930. The use of liver extract was suggested by observation of the frequent marked rise in the leukocyte count in cases of pernicious anemia receiving liver or liver extract. The average increase in the total leukocytes in fourteen cases of pernicious anemia treated with liver was 55 per cent and the average increase in granulocytes was 95 per cent. This tendency to an increase in leukocytes following liver therapy has been recorded by Murphy,[8] Connery and Goldwater[4] and by Conner.[2,3] The rather striking similarity between pernicious anemia and granulocytopenia has been pointed out by

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Fitz-Hugh and Krumbhaar[5] who applied the term "pernicious leukopenia" to this condition. This similarity has been further emphasized by Beck[1] who stated that it seemed reasonable to suppose that the primary lesion is not in the bone marrow but in the organ or tissue which gives rise to the substances that keep maturation of the granulocytes regulated to a normal level. It is possible that the liver is this organ, and that in granulocytopenia there is a deficiency condition as in pernicious anemia. Foran, Sheaff and Trimmer[6] report five cases treated with liver extract given orally, intramuscularly and intravenously. All of these cases recovered. One died in a second attack complicated by pneumonia.

CASE REPORTS

Case 1. In March, 1930, a white woman, age 38, was seen in her home. She had been ill five days. The onset had been with chill, high fever, prostration, extreme nervousness and soreness of the mouth. A blood count made by her attending physician two days before showed 600 leukocytes; the differential formula was not available.

Her past history indicated that her general health had been fairly good until one year before. She was married and had had one child twelve years before that died five days after birth following a difficult labor. She developed bladder irritation in September, 1928, and suffered from this off and on during the fall and winter, with frequency, burning, and blood in the urine. During the winter she was operated on for fissure in ano. In February, 1929, she had an acute attack with chill, high fever, and severe soreness of her mouth. According to her statement her lungs were congested. She was acutely ill for three weeks and continued to feel badly all summer. Her health had been good during the winter months of 1930.

When seen in March, 1930, she appeared extremely ill. She was prostrated and complained of extreme weakness and nervousness. Her temperature had been ranging from 101 to 103°. The skin was pale. There was no icterus of the skin or sclerae. The tongue and the mucous membranes of the mouth were very red. The gums were swollen, tended to bleed, and were covered with a thin, white membrane. There were no areas of necrosis. The neck was somewhat swollen on both sides and the cervical glands were moderately enlarged. There was no ulceration about the rectum or vagina. The spleen was palpable below the costal margin. The general physical examination was otherwise negative. Laboratory findings were as follows: hemoglobin 52 per cent; erythrocytes, 2,270,000; leukocytes, 2,300; polymorphonuclear neutrophiles, 48.0 per cent, small monocytes, 42.0 per cent, large monocytes, 9.5 per cent;

transitionals, 0.5 per cent; platelets, 267,000; reticulocytes, 0.4 per cent; van den Bergh reaction, delayed direct, quantitative 1.8 mg. per liter; blood culture, negative. Smears from the mouth and throat showed no Vincent's organisms. Wassermann test was negative.

The picture at that time did not suggest pernicious anemia but she was given liver extract by mouth, receiving the equivalent of 100 gm. of liver three times a day. There was a rapid improvement in the patient's condition. The mouth cleared promptly, the soreness and swelling of the neck subsided, and the temperature returned to normal in five days. Not much significance was attached to the possible effect of liver therapy at the time, as it was thought that the patient was undergoing a spontaneous recovery. Between March 27, 1930, and July 28, 1930, her erythrocytes gradually increased from 2,270,000 to nearly

TABLE 1

CASE 1

DATE	HEMOGLOBIN	ERYTHROCYTES	LEUKOCYTES	POLYMORPHONUCLEARS	EOSINOPHILES	BASOPHILES	SMALL MONONUCLEARS	LARGE MONONUCLEARS	TRANSITIONALS
1932	*per cent*			*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
April 23*	92	4,690,000	7,000	68.5	3.0	0.5	22.5	4.0	1.5
July 15†	90	4,410,000	1,200	12.0		2.0	62.0	20.0	4.0
July 16			1,060	2.0		0.5	80.0	12.5	5.0
July 18	74	3,640,000	3,500	38.0		0.0	52.5	7.5	2.0
July 20	73	3,500,000	3,700	31.0		0.0	57.5	9.0	2.5
July 26	84	4,420,000	7,600	67.0		1.5	25.5	6.0	

* One vial liver extract every day.

† Three vials, intramuscularly, every day.

5,000,000, her hemoglobin from 52 per cent to about 90 per cent. There were in general no significant blood findings except a myelocyte count of 14 per cent on March 29 followed a few days later by a reticulocyte increase, as high as 10.9 per cent.

The patient continued well during the remainder of 1930, all of 1931, and up to July, 1932, except for one episode in May, 1930, when, following temporary discontinuance of the liver, her leukocytes fell to 3,200, the granulocytes to 44.0 per cent, and the lymphocytes increased to 51.0 per cent. On return of treatment by liver by mouth, the balance was restored. During this time she maintained a normal blood picture on 1 to 2 vials of liver extract each day (hemoglobin, 90 per cent; erythrocytes, 4,500,000 to 5,000,000; differential count, normal.)

On July 15, 1932, the patient stated that for some time she had been having

attacks of generalized aching at intervals of two weeks, accompanied by slight fever, and followed by severe sweats. Two days before she had had such an attack. On examination, her temperature was 99.3°; there was no reddening or ulceration of the gums or throat, and no soreness or enlargement of the glands of the neck. The general physical examination was negative. The blood count, however, showed hemoglobin 90 per cent; erythrocytes, 4,410,000; leukocytes, 1,200; polymorphonuclears, 12.0 per cent; basophiles, 2.0 per cent; small monocytes, 62.0 per cent; large monocytes, 20.0 per cent; transitionals, 4.0 per cent. The following morning her temperature was 101°. The gums were slightly swollen and sore. The glands in the neck were distinctly enlarged and there was general soreness of the neck. The leukocytes numbered 1,060; polymorphonuclears, 2.0 per cent; basophiles, 0.5 per cent; small monocytes, 80.0 per cent; large monocytes, 12.5 per cent; transitionals, 5.0 per cent. The liver extract was increased to three vials by mouth each day, and on the 16th, 18th, and 20th she received 3 cc. of liver extract intramuscularly. The course of her blood response is shown in table 1. It is to be noted that the fall in the leukocytes preceded the drop in erythrocytes and hemoglobin by several days, and that in the restoration of the normal blood picture the same relationship prevailed. This illustrates the importance of the time element in the appearance of anemia in these cases.

From then on she remained in very good health on three vials of liver by mouth each day. In January, 1934, the liver dosage by mouth was reduced to two vials a day. On March 16, 1934, she presented herself complaining of weakness, nervousness, exhaustion, rapid heart action, and soreness of her gums. The gums were red and spongy. There was no atrophy of the papillae of the tongue, and no enlarged glands. The temperature was normal, and the pulse 120. The leukocytes had fallen to 3,000; hemoglobin 76 per cent; erythrocytes, 3,610,000. She was given 3 cc. of liver extract intramuscularly on three successive days and put back on three vials of liver extract by mouth each day. On March 22nd the hemoglobin was 84 per cent; erythrocytes, 4,060,000; leukocytes, 4,300; reticulocytes, 4.2 per cent.

This patient has been observed over a period of four years. The onset of her illness was acute and stormy, with chill, high fever, ulcerative stomatitis, and enlargement of glands of the neck. During this attack her leukocytes fell as low as 600. In one subsequent attack the leukocytes went down to 1,060. In this attack the drop in the leukocytes was detected before the onset of the local symptoms. This attack apparently responded to intramuscular liver extract. In between the acute attacks she had pursued the course of a case of true pernicious anemia. It is worthy of attention that during her initial attack she developed during her period of recovery a myelocytic crisis with 14.0 per cent myelocytes in the blood, and that there was also an increase of her reticulocytes to 10.9 per cent.

Case 2. A white man, age 63, was first seen on February 24, 1932. At that time he had been ill for eight weeks with soreness and stiffness in his joints and the muscles of the legs. He had a normal blood picture: hemoglobin 84 per cent; erythrocytes, 4,170,000; leukocytes, 8,100; polymorphonuclears, 65.5 per cent; eosinophiles, 0.5 per cent, small monocytes, 30.5 per cent; large monocytes, 1.0 per cent; transitionals, 2.5 per cent. His Wassermann and Kahn reactions were positive and he was referred to his family physician for treatment. During March he received three injections of neoarsphenamine; 0.3 gm., 0.45 gm., and 0.6 gm. There was no reaction following any of these injections.

On April 9, 1932, he was seen at home. He had been feeling badly for two days and was complaining of weakness, malaise, fever, and sore throat. On examination the only noteworthy findings were in the throat and neck. The throat was swollen and congested and covered with a dirty, grayish membrane resembling a diphtheritic membrane. Smears taken from the throat were negative for diphtheria bacilli on direct examination and by culture. There was moderate swelling and tenderness on both sides of the neck. The blood count showed 2,900 leukocytes with 2 per cent granulocytes, 76 per cent small mononuclears, 19 per cent large mononuclears, and 3 per cent transitionals. Liver therapy was started at once. At first he was unable to take the extract by mouth on account of swelling and soreness of the throat. He was given, however, 3 cc. of liver extract intramuscularly each day for three days, and then every other day for three doses. There was an immediate subsidence of the swelling and soreness of the throat, return of temperature to normal, and rapid recovery from malaise and weakness. Along with this went an improvement of the blood picture. Over a period of twelve days his leukocytes increased from 2,900 to 6,200, and the granulocytes from 2 per cent to 45 per cent.

During May and June there was some variation in the administration of liver, and both the total leukocytes and the percentage of granulocytes fell again; on June 15, 1932 there were 3000 leukocytes, of which 32 per cent were polymorphonuclear neutrophiles. Following the resumption of liver therapy the blood count reached an approximately normal level in August, 1932. This patient has remained well since that time.

In this case the condition probably arose as a result of arsenic therapy. Clinically and hematologically the primary and secondary cases are indistinguishable and, except for the elimination of the primary factor, the therapeutic indications are the same. This patient pursued a rather chronic course, but it may be of significance that more rapid and complete improvement took place when the liver therapy was better regulated.

Case 3. A white woman, age 39, was admitted to the hospital on July 8, 1933. She had been ill for four days with general malaise, fever, chilly sensa-

tion, exhaustion, and sore mouth and throat. Her temperature had been ranging as high as 103°. She gave a history of what she considered two similar attacks, one four years before, and one two years before. Both of these attacks had been diagnosed as trench mouth. There was nothing else significant in her previous history except that she had lost her husband eight months before and had been very nervous and depressed. As a result of this, during the past eight months she had used sedatives of the barbituric acid series to a considerable extent on account of insomnia.

Examination revealed a well nourished woman, depressed and very nervous. Her color was good. There was extensive ulceration beneath the tongue. The gums were swollen, bluish red in color, and covered with a thin, white membrane that stripped off easily. In two places on the hard palate there were discrete ulcerated areas. The throat was red. The neck was swollen on both sides,

TABLE 2

CASE 3

DATE	HEMOGLOBIN	ERYTHROCYTES	LEUKOCYTES	POLYMORPHONUCLEARS	BASOPHILES	SMALL MONOCYTES	LARGE MONOCYTES	TRANSITIONALS
1933	*per cent*			*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
July 8	84	4,210,000	1,200	18		58	18	6
July 9	90	4,620,000	2,200	28		69	1	2
July 10	89	4,860,000	2,500	24		70	4	2
July 11	88	4,610,000	3,700	53	1	38	6	2
July 12	87	4,460,000	4,000	55		33	7	5
July 13	84	4,410,000	4,500	60		38		2
July 14	85	4,470,000	6,700	54		38	5	3
July 15	84	4,410,000	5,700	70	1	25	3	1

especially the left, which was rather brawny, and the glands at the angle of the jaw were enlarged on both sides. The neck was very tender to palpation. The head was normal, the lungs clear, and the spleen not enlarged. The vaginal tract was negative.

On admission her temperature was 101°, on the second day 102, and on the third day was normal and remained so thereafter except for a slight afternoon rise for two days. The blood examination on admission showed a leukocyte count of 1,200 with 18 per cent granulocytes. The subsequent course of the blood counts is shown in table 2. Blood culture was negative. Smears from the mouth showed no fusiform bacilli. She was given 3 cc. of liver extract intramuscularly and put on liver extract by mouth three times each day. The second day she received two injections of liver extract, the third day one, and thereafter was continued on the extract by mouth. By the seventh day the blood picture had returned to normal and remained so thereafter. With the

fall in temperature and improvement in the blood picture, there was a rapid subsidence of symptoms. The mouth cleared; the swelling and tenderness in the neck disappeared and there was an immediate return of a sense of well-being.

This patient made a very rapid recovery while taking liver by mouth and intramuscularly. While the change in the blood picture was rapid and striking, the clinical improvement was even more impressive. The history would indicate that she had experienced two other attacks from which she recovered spontaneously, neither one of these, however, was as severe as the last attack. It is possible that she again recovered spontaneously, but the rapid change in her clinical condition following liver therapy strongly suggested a causal relation. There has been no opportunity to observe this patient since discharge from the hospital eight days after admission, but a recent communication from her doctor indicates that she has remained well.

The three cases reported have recovered and are apparently well at the present time. One of them has to continue on a regular ration of liver. If this is diminished or discontinued she tends to revert, not only to a pernicious type of blood picture, but to a leukopenic state as well.

It is, of course, realized that in reporting results in these three cases no very valid conclusions can be drawn. It is possible that all of these cases would have made spontaneous recoveries without the aid of liver extract. It is felt, however, that the results obtained are suggestive and would seem to justify the further use of liver extract in the treatment of granulocytopenia.

REFERENCES

(1) Beck, R. C.: Benign and malignant neutropenia. Arch. Int. Med., **52:** 239–287. 1933.

(2) Conner, H. M.: Injection of liver extract in the treatment of pernicious anemia. Jour. Am. Med. Assn., **99:** 614–620. 1932.

(3) Conner, H. M., Margolis, H. M., Birkeland, I. W., and Sharp, J. E.: Agranulocytosis and hypogranulocytosis. Abstr. in Jour. Am. Med. Assn., **98:** 1220. 1932.

(4) Connery, J. E., and Goldwater, L. J.: Parenteral use of liver extract in treatment of pernicious anemia. Jour. Am. Med. Assn., **98:** 1060–1067. 1932.

(5) Fitz-Hugh, Thomas, Jr., and Krumbhaar, E. B.: Myeloid cell hyperplasia of the bone marrow in agranulocytic angina. Am. Jour. Med. Sci., **183:** 104–110. 1932.

(6) Foran, F. L., Sheaff, H. M., and Trimmer, R. W.: Agranulocytic angina. Jour. Am. Med. Assn., **100:** 1917–1918. 1933.

(7) Friedemann, U.: Über Angina agranulocytotica. Med. Klin., **19:** 1357–1359. 1923.

(8) Murphy, W. P.: The parenteral use of liver extract in pernicious anemia. Jour. Am. Med. Assn., **98:** 1051–1060. 1932.

(9) Schultz, Werner: Ueber eigenartige Halserkrankungen. Deut. Med. Wchnschr., **48:** 1495–1596. 1922.

EDITORIAL

The Passive Transfer Method of Allergic Diagnosis

During the last ten years there has been a very definite tendency toward the specialization of allergic practice. A variety of factors have made this necessary. Allergy is a rapidly growing new branch of medicine in which the advances have been made chiefly by those investigators who have had the opportunity to devote practically their entire time to the subject. At first it appeared as if allergic diagnosis would be a very simple laboratory procedure in which certain tests were applied directly to the patient's skin and the answer was thereupon correctly revealed to anyone who chose to read the reactions.

So many variables, however, play a part in the elicitation of the skin reaction that this is no longer considered, in any sense, infallible. Many supplementary methods of investigation have since been developed and it is quite true that for the adequate study of the allergic diseases, one's full time is often required.

At the same time allergy has been found to dominate or to color such a wide variety of clinical manifestations and such a relatively large proportion of the population that the time will come when the man not primarily interested in allergic investigations must give certain of his patients the benefits of allergic study. While, today, there is scarcely room for argument that the investigator who performs the skin tests should be the person who is making the entire allergic study in the broader sense, at the same time convenience or necessity often dictates that the general man have this portion of the allergic work done by someone appropriately equipped therefor. As a consequence in certain sections, the clinical pathologist is called upon to do the routine sensitization tests, reporting the results of study to the attending physician.

Although the direct method of testing usually gives a large

amount of positive information and remains at present the basic point of departure for allergic studies, it's shortcomings may be briefly summarized under three categories: the appearance of a certain proportion of false positive reactions, reactions to allergens which are subsequently shown to have no clinical significance in the particular case under investigation; false negative reactions, in which for one reason or another information that should have been of value was not elicited; and inappropriate reactive surface, in which the patient's skin for one reason or another cannot be used. This occurs especially in infants, in individuals with extensive dermatitis, in cases of dermographia, in individuals too ill to be subjected to the inconvenience of skin testing, and in those who find it necessary to take adrenalin so continuously that the skin reaction is not reliable.

One of the several methods available for counteracting any or all of these disadvantages is the passive transfer method, which, incidentally, is truly a laboratory procedure. This method has been developed on the basis of the familiar Prausnitz-Kustner reaction, in which antibodies or reagins may be transferred from the serum of an allergic individual into the skin of a non-allergic recipient, thereby rendering this skin area sensitive for a short time to the particular allergen in question. The method has been popularized in this country by Matthew Walzer who describes it in detail in "Asthma and Hay Fever in Theory and Practice"* and in a number of subsequent contributions.

Briefly the method consists in obtaining 5 or 10 cc. of the patient's blood under aseptic precautions, separating serum or preferably plasma; ultra-filtration; and its introduction intracutaneously into selected skin areas of a non-allergic recipient. Walzer uses no preservative although the writer has found no disadvantage to the addition of 0.5 per cent phenol. The sensitizing dose should be rather large (0.07 cc. to 0.1 cc.). An area of perhaps two inches diameter becomes sensitized and remains so for upwards of four weeks. Walzer recommends the arm, the

* Coca, A. F., Walzer, Matthew, and Thommen, A. A.: Asthma and hay fever in theory and practice. Springfield: Thomas, 1931, pp. 851.

writer prefers the back. Usually a mole or other landmark is available as a point of orientation, from which one side of the back is checker-boarded with the intracutaneous injections, two inches apart vertically and horizontally. Twenty-five or more areas may be sensitized.

The trauma of the procedure is apt to produce a refractory period which may last twenty-four or forty-eight hours, or longer. By preference the actual testing is therefore not done until the third or fourth day after preparation.

At this time the test solutions are introduced, in the usual 0.01 cc. or 0.02 cc. dosage, at points about one quarter inch from the sites of the original needling. Control tests are applied in the same dosage at symmetrical points on the non-sensitized half of the back.

Readings are made usually at the end of twenty or thirty minutes. Each test substance is read by comparison with its control reaction. Although, occasionally, large urticarial reactions result, any real degree of difference from the control is of significance. Not infrequently some of the control reactions are positive, indicating either an unsuspected sensitiveness on the part of the recipient, or an irritating test solution. Such reactions cannot be interpreted and should, if feasible, be repeated on another recipient. When a control reaction is positive and the passively sensitized area is very much more strongly positive, this is usually indicative of a positive reaction.

After an additional three or four days the same areas may again be used for further testing. Areas that have given positive reactions on the first testing should however not be used again, and most particularly, should not be used with the same test solution that gave the original positive reaction, since the antibodies have been exhausted for this particular antigen.

The advantages claimed by Walzer for this method are as follows: so-called false positive reactions are avoided since the reacting body must be present in the patient's blood for the reaction to be positive by this method; the method may be used in cases in which the condition of the patient's skin precludes satisfactory direct testing, such as in diffuse eczema with or without

secondary infection, icthyotic skin, and those persons scarred by constant scratching in inflammation; urticaria, dermographism; contagious skin infections such as impetigo; and in the other conditions mentioned above.

—Warren T. Vaughan.

NEWS AND NOTICES

Announcement has been received of the organization of the American Association for the Study of Neoplastic Diseases. It is planned that the Society will hold four meetings a year and that the September meeting will be held at the Mayflower Hotel in Washington, September 6 to 8. The program consists of two sessions with lantern slide demonstrations of microscopic pathology, two sessions on radiologic diagnosis, and three sessions on surgery and radiation therapy in neoplastic diseases. All persons interested in the work of the Association are invited to attend the meeting.

Dr. M. Pinson Neal served as Visiting Professor of Pathology and Bacteriology at the University of Tennessee College of Medicine and as Acting Pathologist to the Memphis General Hospital during the summer quarter at Memphis, Tennessee.

The International Society of Radio-Biology will sponsor an International Congress of Electro-Radio-Biology to be held in the Doges Palace in Venice, September 10 to 15. The Congress will be presided over by Marquis Guglielmo Marconi. The object of this Congress is to invite for a discussion, physicists, chemists, biologists, naturalists, and physicians, on biological actions of all radiations, in order to coördinate the respective investigations. A general invitation has been made to all others interested in the subject.

The Serologic Conference

The Society will be interested to learn that Surgeon-General Cumming of the United States Public Health Service has included the "serologic conference" in the budget of that government service for the coming year. The committee that reported on this matter at the Cleveland meeting was continued with power

to act. Accordingly, Dr. Vonderlehr of the U. S. P. H. Service was made chairman with Drs. Simpson and Sanford on the committee to represent the A. S. C. P. Two syphilologists are to be chosen by these three members and this committee of five will have charge of the plan to evaluate independent serologic procedure for the diagnosis of syphilis in the United States.

The following notice is to be published in various medical journals:

The United States Public Health Service is coöperating with the American Society of Clinical Pathologists in the drafting of a plan to evaluate independently serologic procedure for the diagnosis of syphilis in this country. Briefly, the plan contemplates the collection of specimens of blood from at least 1,000 individuals and the distribution of comparable specimens to the laboratories of serologists who have described an original modification of a complement-fixation or precipitation test for the diagnosis of syphilis. The donors of the specimens will be carefully selected so as to measure both the specificity and sensitivity of the serologic procedure. The sending of specimens to workers at considerable distance from the point of collection will be expedited by the use of the most modern transportation facilities, while the delivery of specimens to nearby serologists will be delayed so as to make the delivery time approximate that for those workers at the more remote points.

A committee of five members consisting of two specialists in the field of clinical syphilology, two members of the American Society of Clinical Pathologists, and one officer of the United States Public Health Service will organize the plan of study and, after all laboratory reports have been submitted by participating serologists, will interpret the results on the basis of clinical findings. The collection of the specimens will begin about December 1, 1934, and a number of serologists will be invited to take part in the evaluation scheme.

It is possible that the name of some serologist who has described an original modification of a test for syphilis may have been inadvertently omitted. Any serologist desiring to participate will be extended an invitation upon presentation of suitable proof

as to the originality of his modification of a serologic test. A brief description of the plan will also be sent to those workers who may be interested.

Correspondence should be addressed to the Surgeon-General, United States Public Health Service, Washington, D. C.